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THE TOTAL METABOLISM OF RATS DURING FASTING AND REFEEDING

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It is well known that the total energy metabolism of animals falls during fasting (e.g. Tigerstedt, 1910; Lusk, 1931). On the other hand, the available data demonstrate much disagreement on the extent of the change in either the total or the basal metabolic rate (M.R. and B.M.R.) during fasting.

If an animal is starved for a period it loses weight, and its metabolism can be expected to fall by an amount corresponding to (but not necessarily proportional to) the decrement of active body tissue. Where the total metabolism is measured, the fact that the animal is deprived of food reduces its heat production also by reducing its activity and the heat increment due to obtaining, eating, digesting and metabolizing food. While, however, the energy cost of eating is largely eliminated in complete deprivation of food (although refection and rumination may continue in some animals), the other components of the heat increment of feeding are not completely eliminated and may, in certain circumstances, increase. The question arises, therefore, whether the fall in total energy expenditure during fasting can be entirely accounted for by the changes in metabolizing mass and food intake, or whether there is also a component accountable to fasting qua fasting; i.e. whether there is a true change in metabolic rate when changes in body weight and in 'supra-basal' energy costs are included in the reckoning.

The analysis of this type of problem is always complicated by difficulty in taking body size into account. Body size is most conveniently measured as body weight, and that has been used in this work. Many other measures of body size have been suggested as reference bases for metabolic rate, but they have never been shown to be applicable to total metabolism. When body weight, or any function of body weight, is used as a measure of body size, then, if the decrease of each of the chemical components of body weight is in proportion to the corresponding concentration of each component in the normally fed animal, it would be reasonable to expect the decrements of body weight and of metabolizing potential to be proportional. If, however, the components alter in different proportions, then it is not strictly legitimate to equate the fall in metabolizing mass with fall in body weight. It is apparent that any resultant change in the manifest

metabolic rate of the animal could as easily be due to a change in ratios of more rapidly to more slowly metabolizing tissues, as to a change in metabolic rate of one or more tissues.

It is not possible, at present, to acquire all the information necessary to answer, for the whole animal, the absolute question: is there a change in metabolic rate of tissues during fasting? It is, however, possible, to ask whether there is any change in the total energy metabolism of a fasting animal independent of changes which lie in the empirical relationship between energy metabolism on the one hand and body weight and the heat increment of feeding on the other. This form of question makes no assumption about the composition of the decrement of body weight, or about changes in the individual components of energy expenditure. The methods here used allow some analysis of the composition of body weight change and of the degree of variation in activity of those animals whose energy expenditure is measured, during the precise periods for which it is measured. The manifest behaviour of the total metabolism has been examined in the light of these changes.

METHODS

General

The work was done on five adult male hooded rats of initial body weight 180–260 g. A preliminary experiment was done on one rat for three experimental periods. Several technical problems and faults arose in this experiment which limited the validity of the findings; these are in substantial agreement with the remainder of the work but they have not been included in the general analysis of the results. The main experiment was done on four rats, each of which was subjected to two periods of fasting and recovery.

The experimental periods all consisted of 2 days' normal feeding with water and food (diet 41, Bruce & Parkes, 1949) available *ad lib.*, 2 days' total deprivation of food (but no restriction of water intake), and some days' recovery with food and water *ad lib.* The recovery periods were, for the first rat, 2, 3 and 1 days in the successive experimental periods, and 4 days for each experimental period in the other 4 rats (main experiment).

The animals were maintained in an indirect calorimeter for each 24 hr of the experimental 8-day period. The technique has been described in detail previously (Morrison, 1955). Body weight was measured daily. The total oxygen consumption, carbon dioxide production, food and water consumption, urine weight, vaporized water, faecal mass, and urinary, faecal and food nitrogen were measured for each 24 hr of experiment. Faecal and food energy were directly measured by bomb calorimetry. The environmental temperature in the animal chamber during experiment was 23–25° C and the average relative humidity was 40%.

Derived values, such as energy expenditure, were computed as described previously (Morrison, 1955). The total respiratory quotients (R.Q.) were calculated from the total oxygen consumption and carbon dioxide production for each 24 hr. For non-protein R.Q. the correction for protein catabolism was calculated from the measured urinary nitrogen for each 24 hr. The amounts of urine solids and of water of urine were calculated for each day of experiment, from the measured 24 hr nitrogen, from a relationship established experimentally as part of later work:

$$S = (0{\cdot}174 \pm 0{\cdot}05) + (0{\cdot}004 \pm 0{\cdot}0003)\,U, \qquad (1)$$

where S is solids of urine in grams and U is total nitrogen of urine in milligrams.

During starvation the normal methods of indirect calorimetry in mammals are thrown into error by the amount of ketone bodies appearing in the urine and expired air. The rat, however, shows only a slight fasting ketosis. The elimination of ketone bodies by the rat on the second day of fast in the urine (Roberts & Samuels, 1949; Mayes, 1959) and in the expired air (Pratt, Burr, Eden & Lorenz, 1951) amounts to less than 0·1 % of the daily turnover of energy.

Partition of body-weight increments

Any body-weight increment consists of a part attributable to change in tissue mass and a part attributable to change in 'extra-corporeal' mass, notably (and for dry matter, almost entirely) to change in gut contents. Average values for the amounts of each can be obtained by slaughter and chemical analysis, but this does not give useful information about the changes in one animal. Composition of carcass shows great variation between individual animals of equal body weight or of equal age and maintained as nearly as possible under identical conditions (Pratt & Putney, 1959; Cumming & Morrison, unpublished work). Variation in mass of gut contents between individual animals maintained under control conditions is as great as the change caused by imposition of a 48 hr fast (Table 1). A method of deriving, separately, for an individual animal throughout experiment, the increments in body tissue and gut contents would, therefore, be of value. If complete figures are available for energy, nitrogen, water and weight balances it is possible to partition the dry matter, nitrogen and energy increments into tissue and gut components. Use of average direct measurements of moisture in gut contents (which is less variable than total gut contents) enables the partition to be made, although less accurately, for water.

The total retention of dry matter by an animal over any period can be expressed in the form:

$$\triangle F + \triangle P + \triangle C + \triangle M + \triangle R + \triangle G = \triangle \text{wt.} - \triangle H_2O, \tag{2}$$

where $\triangle F$, $\triangle P$, $\triangle C$ and $\triangle M$ are the tissue increments of fat, protein, carbohydrate and minerals, respectively, $\triangle R$ represents the tissue increment in other dry matter, $\triangle G$ is the dry-matter increment of gut contents, and $\triangle$wt. and $\triangle H_2O$ are the total increments of body weight and of body water.

It can be assumed that $\triangle M$ and $\triangle R$ are negligible over a 24 hr period. The values for the 24 hr non-protein R.Q. (Fig. 2*a*) show that $\triangle C$ is only likely to be large on the first day of fast and on the first day of refeeding. These changes in $\triangle C$ are likely to be about $\pm 0{\cdot}5$ g or 2·1 kcal (estimated from Cori & Cori, 1926; Mayes, 1959).

If $\triangle G$ could also be assumed to be zero there would be two ways of calculating the changes in tissue fat during the period:

$$\triangle Fat' = \triangle \text{wt.} - \triangle H_2O - 6{\cdot}25 \triangle N \pm \triangle C, \tag{3}$$

$$\triangle Fat'' = (\triangle Energy - 35 \triangle N \pm 4{\cdot}2 \triangle C)/9{\cdot}45, \tag{4}$$

where $\triangle Fat$ is the apparent increment of body fat, $\triangle N$ and $\triangle Energy$ are the measured total increments of nitrogen and energy. The constants 6·25, 35 and 9·45 in these equations are, respectively, the conversion factor for nitrogen to tissue protein (assuming tissue protein to contain 16 % nitrogen), the heat of combustion of protein in kcal/g protein nitrogen and the heat of combustion of tissue fat in kcal/g fat. The value of 35 kcal/g protein nitrogen for a protein containing 16 % nitrogen implies a heat of combustion of 5·6 kcal/g for protein. These are close to the values assumed in the calculation of heat production from the respiratory and nitrogen exchange (Loewy, 1911).

The value for $\triangle C$ in equations (3) and (4) has been taken as $-0{\cdot}5$ g for day 3, $+0{\cdot}5$ g for day 5 and zero for all other days. The mean fat increments estimated by these equations for each day of experiment are shown in rows 1 and 2 of Table 1. The most notable discrepancies between the two methods of derivation of apparent $\triangle Fat$ occur for the first day of fasting (day 3) and for the first day of refeeding (day 5). The discrepancies arise predominantly from the difference between the energy values of fat and of gut contents.

To partition the increment of dry matter between tissue and gut contents, energy values of 9·45 kcal/g tissue fat (Brody, 1945) and of 4 kcal/g protein-free dry matter of gut contents (mean of heats of combustion of food and of faeces with allowance made for nitrogen content) have been assumed. The value of 4 kcal/g for protein-free dry matter of gut contents is a composite one, as the gut contents are made up of carbohydrate, fat and non-energy-providing material. This value will, therefore, alter with changes in composition of the diet, with changes in the absorption of the diet from the gut and with changes in distribution of gut contents between stomach and intestine. Because of the great variation in distribution of gut contents in normal animals, directly measured values obtained later for heats of combustion of protein-free stomach contents (4·00 kcal/g) and protein-free intestinal contents (3·95 kcal/g) were considered not to give a useful improvement in precision.

TABLE 1. Average values for two runs on each of four male hooded rats*

	Pre-fasting period		Fasting		Re-feeding			
Days	1	2	3	4	5	6	7	8
△ *Fat'* (g)†	+0·95	+0·70	−4·51	−3·14	+3·68	+1·33	+1·61	+2·09
△ *Fat''* (g)‡	+0·67	+0·73	−3·18	−2·72	+1·98	+1·45	+1·45	+1·74
D.M. increment in gut contents (g)§	+0·58	−0·08	−2·96	−1·03	+3·60	−0·24	+0·34	+0·75
D.M. increment in tissue (g)‖	+0·77	+0·98	−3·54	−3·33	+1·27	+1·59	+1·71	+1·99
% H_2O assumed in increment in gut contents	78·5	78·5	78·5	78·5	65·0	68·0	73·0	78·5
Direct measurement of gut contents								
Mean *D.M.* (g)	2·22		0·94	1·29	3·86	2·21	—	4·82
Range, *D.M.* (g)	0·9–5·3		0·6–1·2	0·9–2·0	2·2–7·3	1·8–2·5	—	3·4–6·9
% H_2O (range)	66–81		77–81	75–82	63–76	70–84	—	69–76
No. of animals	9		3	3	6	3	—	3

D.M. = dry matter. * Day 5 values are the means of 7 sets of observations. † As estimated by equation (3). ‡ As estimated by equation (4). § As estimated by equations (5)–(8). ‖ Difference between total *D.M.* increment and *D.M.* increment of gut contents.

The following equations can be constructed:

$$\triangle D = g + t \pm \triangle C \tag{5}$$

and

$$\triangle H = 4g + 9{\cdot}45t \pm 4{\cdot}2\triangle C, \tag{6}$$

where $\triangle D$ is the total non-protein dry-matter increment, $\triangle H$ is the total non-protein energy increment, g is the non-protein dry-matter increment of gut contents and t is the non-protein dry-matter increment of tissue. These simultaneous equations were solved for each 24 hr of experiment.

The dry matter of gut contents, estimated in this way, is increased to take account of the nitrogen of gut contents. For all non-fasting days this nitrogen concentration has been taken as the mean of nitrogen of dry food and of dry faeces, 29 mg/g. For the first and second days of fast the nitrogen of gut contents has been taken as the nitrogen of dry faeces on each day, 36 and 50 mg/g, respectively. To find the total dry-matter increment of gut contents the following equations are used:

$$D.M. = g + 6{\cdot}25N, \tag{7}$$

where

$$n = N/(g + 6{\cdot}25N), \tag{8}$$

where *D.M.* is total dry-matter increment of gut contents in grams, N is total nitrogen increment of gut contents in grams, and n is nitrogen content of dry matter of gut contents in g/g. The increment of tissue nitrogen is derived as the difference between total nitrogen increment

and nitrogen increment of gut contents; the increment of tissue dry matter is derived as the difference between ($\triangle$wt. $- \triangle H_2O$) and the dry-matter increment of gut contents. This calculation was made for each day of experiment on each rat. The mean dry-matter increments of gut contents and of tissue for each day of experiment are given in lines 3 and 4 of Table 1.

The parallel calculations to divide total water increment into tissue water and water of gut contents require values for the moisture content of gut contents. This can only be obtained from average values for water content of gut contents of killed animals, and is, therefore, more liable to error than the estimation of dry-matter increments. Two groups of 15 and 12 rats were subjected to the same sequence of fasting and refeeding on the same diet. In all, 9 animals were killed after 3 days in individual cages on an *ad. lib.* diet, 3 were killed after 1 day of fast, 3 after 2 days of fast, 6 after 1 day of refeeding, 3 after 2 days of refeeding and 3 after 4 days of refeeding. The rat was killed by a blow on the head, the stomach and intestinal contents were removed immediately, were weighed separately and were then dried to constant weight. The moisture contents of gut contents obtained from these rats have been applied to the dry-matter increments in gut contents for each day of the metabolic experiments and are shown in row 5 of Table 1. The dry matter of the gut contents so measured is also shown in Table 1.

RESULTS

Energy exchange

Expenditure of energy. In all experimental periods the total heat production fell rapidly during the 2 days of fasting to 78 % of the pre-fasting production, and recovered, during the refeeding period, by progressively smaller increments (Fig. 1*a*). In only 3 out of the 8 experimental periods did the energy expenditure completely regain its initial (pre-fasting) level within the 4 days of refeeding. The mean recovery by the end of the fourth day of refeeding was to 95·9 % of the initial expenditure.

Body weight fell continuously during the fast, to 90·5 % of the initial value, and recovered during the refeeding period to 97·3 % of the initial body weight (Fig. 1*b*). The recovery of body weight by individual animals was rather irregular (Table 2). Only 71 % of the weight lost during fasting was recovered during refeeding, compared with a recovery of 81 % for energy expenditure.

Food intake during the refeeding period was consistently higher than during the pre-fasting period (Fig. 1*c*). The mean increase in gross food intake was 14·5 % ($P < 0{\cdot}001$).

Analysis of co-variance of the values for energy expenditure with those for body weight and for apparent absorbed food energy (Tables 2 and 3) allows the adjustment of the daily means to eliminate changes accountable to changes in body weight and food intake. The regression of energy expenditure on body weight and apparent absorbed energy intake (total energy intake less faecal energy) accounted for 99 % of the variation between days of experiment; no significant variance remained after removal of the multiple regression component ('days + residual' in Table 3;

$P > 0{\cdot}1$). The adjusted line for total energy expenditure at a constant body weight of 240 g and constant daily apparent absorbed energy of 50 kcal is shown in Fig. 1*a*. The equation to the multiple regression between days of experiment (and including residual) is:

$$E = (0{\cdot}24 \pm 0{\cdot}02)\,W + (0{\cdot}055 \pm 0{\cdot}006)\,N - (22{\cdot}1 \pm 4{\cdot}4), \qquad (9)$$

TABLE 2. Values used for the analysis of co-variance of energy expenditure, body weight and food intake

		Pre-fasting period		Fasting		Refeeding			
Days...		1	2	3	4	5	6	7	8
Rat no.	Run								
		Energy expenditure (kcal/rat/24 hr)							
1	a	36·5	38·8	32·7	29·1	33·5	35·4	34·9	35·7
	b	39·6	39·8	32·9	29·3	33·2	35·7	36·7	36·9
2	a	39·8	39·1	35·4	31·3	34·7	36·9	36·7	39·5
	b	40·5	39·9	35·2	31·9	37·5	37·0	39·0	38·1
3	a	47·0	47·6	40·7	35·0	40·9*	40·1	40·9	43·7
	b	42·1	43·5	37·4	35·9	37·2	41·4	42·2	39·8
4	a	32·4	33·3	28·1	25·3	31·2	28·7	32·5	33·1
	b	31·8	29·2	24·7	24·0	28·4	28·7	29·2	30·6
Mean		38·7	38·9	33·4	30·2	34·6	35·5	36·5	37·2
		Mean daily body weight (g)							
1	a	232·1	233·2	223·4	210·7	213·2	223·9	226·7	225·5
	b	251·0	252·0	242·8	227·9	231·2	241·6	244·1	244·8
2	a	255·1	256·3	248·0	234·1	236·0	244·8	248·2	251·1
	b	273·4	273·4	263·7	249·4	252·5	259·9	260·2	262·7
3	a	260·8	259·0	249·8	236·1	237·4	248·3	253·0	256·3
	b	274·4	272·5	265·9	249·6	251·3	262·7	265·9	265·6
4	a	179·7	178·2	167·6	161·4	164·6	165·1	166·4	178·6
	b	182·3	180·6	172·7	159·8	159·7	166·7	168·5	171·5
Mean		238·6	238·1	229·2	216·1	218·2	226·6	229·1	232·0
		Mean daily body weight calculated with fasting gut contents (g)							
1	a	212·9	210·2	210·2	208·5	208·3	213·3	214·3	207·3
	b	231·8	230·1	228·9	224·9	225·2	230·3	233·6	235·5
2	a	237·9	235·3	232·3	231·5	231·3	236·4	238·3	236·7
	b	250·8	250·3	249·2	245·4	245·5	248·3	249·5	248·6
3	a	249·7	249·5	245·6	234·7	231·9	237·5	240·2	240·9
	b	246·1	243·2	244·9	245·1	247·6	256·0	263·9	270·1
4	a	175·1	170·7	168·3	161·7	156·7	150·3	147·6	150·5
	b	154·4	151·5	155·5	157·5	157·8	163·0	165·3	166·7
Mean		219·8	217·6	216·9	213·7	213·0	216·9	219·1	219·5
		Apparent absorbed energy intake (kcal/24 hr)							
1	a	60·2	48·8	−5·9	−0·8	66·5	63·2	49·6	63·8
	b	64·5	65·9	−6·4	−1·2	64·8	57·7	58·4	59·3
2	a	41·7	56·3	−8·5	−0·4	61·5	63·7	60·2	61·7
	b	46·5	39·8	−5·8	−1·0	71·9	49·4	59·1	61·1
3	a	62·9	52·2	−5·0	−0·6	67·0	59·3	58·0	63·8
	b	44·4	60·9	−6·0	−0·9	62·5	66·9	60·1	58·1
4	a	36·9	36·8	−2·0	−0·9	51·4	29·7	50·0	54·1
	b	36·5	33·6	−4·0	−0·8	43·9	44·1	42·5	51·9
Mean		49·2	49·3	−5·5	−0·8	61·2	54·3	54·7	59·2

* This is a fitted value. The measured gaseous exchange for that day (giving a value of 34·2 kcal) showed evidence of error.

where E is total energy expenditure in kcal/24 hr, W is the body weight in grams and N is the apparent absorbed food energy in kcal/24 hr.

The simple regression on body weight accounted for only 82% of the variation between days, leaving a significant residual ($P < 0{\cdot}001$) (Table 3). The energy expenditure at constant body weight, adjusted by the simple regression on body weight (Fig. 1*a*), shows a fall in metabolic rate on the first day of fasting, and a rise above the pre-fasting level during the first day of refeeding.

TABLE 3. Analysis of co-variance of energy expenditure (E) with observed body weight (W) and absorbed energy intake (N), and with calculated empty body weight (C) and absorbed energy intake (N)

	Multiple regression on W and N			Multiple regression on C and N			Regression on W alone		
Source of variance	Variance regression D.F. = 2	Variance error	D.F. error	Variance regression D.F. = 2	Variance error	D.F. error	Variance regression D.F. = 1	Variance error	D.F. error
Residual*	4·7	1·32	46	4·4	1·34	46	2·3	1·44	47
Days + Residual†	234·3	1·35	53	141·7	4·84	53	351·2	3·50	54
(Days + Residual)* − Residual	229·6	2·13	5	137·3	39·05	5	348·9	20·17	6
Runs + Residual	476·7	4·95	53	483·6	4·69	53	939·5	5·12	54
(Runs + Residual) − Residual	472·0	40·3	5	479·2	37·41	5	937·2	34·73	6
Total	670·6	5·74	60	626·9	7·20	60	1152·1	8·75	61

D.F. = number of degrees of freedom. *These lines are compared to obtain the significance of the residual variation between days. †The regression coefficients derived from this component (equations 9 and 10) have been used to adjust the daily energy expenditure to constant body weight and constant food intake (Fig. 1*a*).

Energy expenditure with 'constant' gut contents. The increments in gut contents for individual days of each experimental run were calculated as described in Methods. Using these figures and starting from the total observed body weight at the end of the second day of fast, we have derived values for the body weight assuming a constant gut content (that at the end of the second day of fast). These derived body weights incorporate all the errors involved in estimating both dry matter and water increments of gut contents, and it is apparent (Table 1) that the gut contents after 48 hr fasting show considerable variation. The absolute values for the derived body weights are thus not very accurate. Undoubtedly, however, they represent much more closely the true variations in metabolizing tissue than do the observed body weights. The multiple regression relationship between energy expenditure and this adjusted body weight and food intake ('days + residual', Table 3), is:

$$E = (0{\cdot}113 \pm 0{\cdot}047)C + (0{\cdot}073 \pm 0{\cdot}011)N + (8{\cdot}2 \pm 10{\cdot}3), \qquad (10)$$

where C is the body weight in grams with fasting gut contents. E and N are as previously. The residual variance after removal of the multiple

regression was highly significant ($P < 0{\cdot}001$) (Table 3). The energy expenditure adjusted by the coefficients of equation (10) to constant 'empty' body weight and constant food intake (Fig. 1*a*), shows a drop in total metabolic rate during fasting with slow recovery during refeeding.

Sources of energy expenditure. During the pre-fasting period 78, 6·5 and 15·5 % of the energy expenditure were derived from carbohydrate, fat and

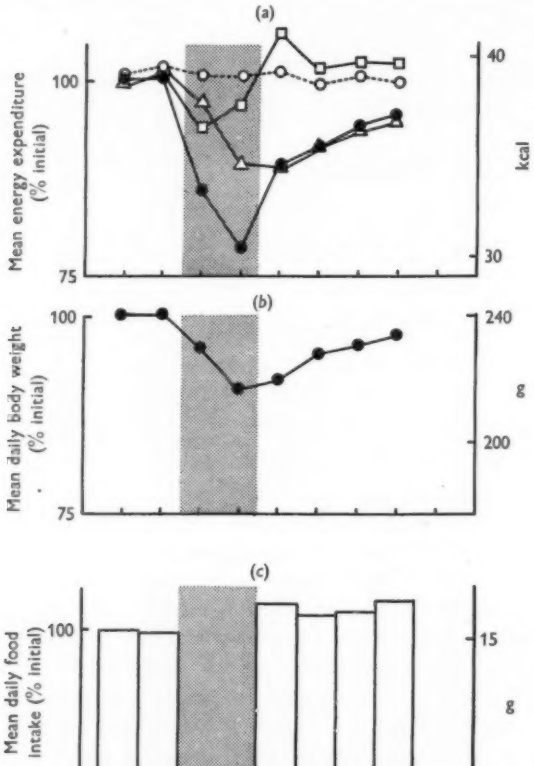

Fig. 1. Changes in energy expenditure, body weight and food intake throughout the experimental period. (*a*) ●—● mean daily energy expenditure; □—□ mean daily energy expenditure adjusted to 240 g body weight with no account taken of food intake; ○- - -○ mean daily energy expenditure adjusted to 240 g body weight and 50 kcal absorbed energy intake; △—△ mean daily energy expenditure adjusted to 220 g empty body weight and 50 kcal absorbed energy intake. (*b*) ●—● mean daily body weight. (*c*) Each block represents mean daily food intake. In all figures stippled area represents the fasting period.

protein respectively (Fig. 2*b*). The non-protein R.Q. (Fig. 2*a*) for this period was 0·977 compared with the theoretical value for the diet of 0·955. The effect of this is seen in the tissue gain of energy from fat during this period (Fig. 2*c*).

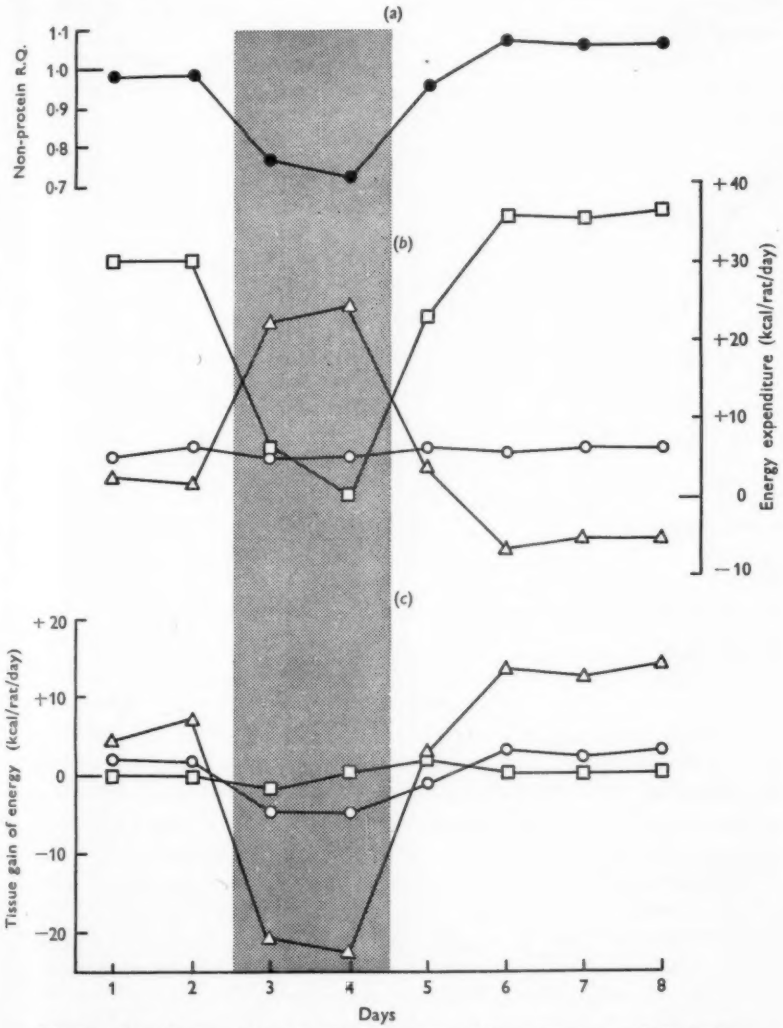

Fig. 2. (*a*) ● Non-protein R.Q. (*b*) Energy expenditure from □ carbohydrate, △ fat, ○ protein. (*c*) Tissue increment of energy from □ carbohydrate, △ fat, ○ protein.

During the first day of fasting the R.Q. fell to 0·766 and the derivation of energy was altered to 17·5, 67 and 15·5%. During the second day of fasting the R.Q. fell to 0·714 with the derivation of energy 2·5, 81 and 16·5% from carbohydrate, fat and protein respectively. The change to catabolism of fat during this period is shown by the calculated tissue increment of energy from fat (Fig. 2*c*).

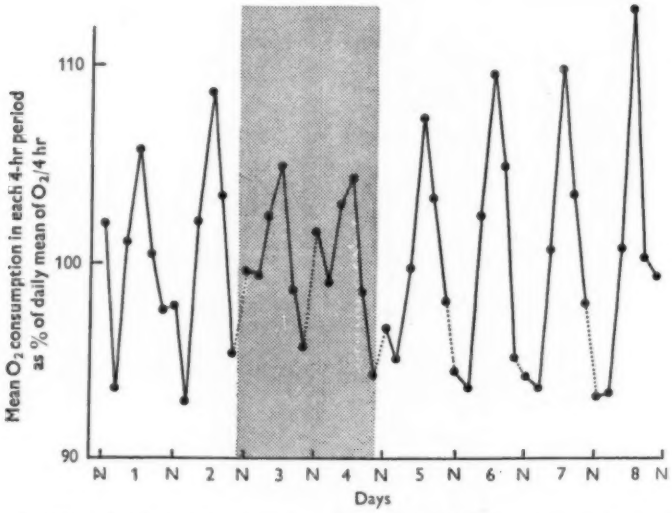

Fig. 3. Diurnal variation in oxygen consumption. ● Mean consumption for each 4-hr period of experiment expressed as a percentage of the mean 4-hr consumption within the day in which the 4-hr period occurred; - - - - - - contain the times at which daily weighings and other procedures were done. N, noon.

On the second day of refeeding the R.Q. rose to a mean of 1·054 and remained above unity for the rest of the experimental period. Out of the 24 individual daily values for the last 3 days of refeeding only 2 were below unity. The mean energy derivation during this period was 101, −17 and 16% from carbohydrate, fat and protein respectively.

Diurnal variation in oxygen consumption. This was found from the total oxygen consumption over each 4-hr period throughout the day and night as described previously (Morrison, 1955). The mean 4-hr oxygen consumption for each 24 hr of experiment has been expressed (Fig. 3) as percentage of mean 4-hr intake throughout that 24-hr period.

The cyclical variation throughout the 24 hr was apparent as before (Morrison, 1955, 1956). The amplitude of the variation during the pre-fasting period was 17% of the mean 4-hr consumption, and during the

fasting period was 14 %. These mean amplitudes do not differ significantly (> 0·1). During the first day of refeeding the diurnal variation did not alter, but during the last 3 days of refeeding recorded it rose to 23 %, significantly higher than all previous values ($P = 0{\cdot}001$). There was no consistent shift in time of maximum and minimum oxygen consumption during the experimental period.

Faecal energy loss (Fig. 4). The dry matter of the faeces was only 0·2 g on the second day of starvation, compared with the pre-fasting level of 3·7 g. The heat of combustion per gram of faecal dry matter during the fasting period was 4·17 kcal, slightly greater than that in the pre- and post-fasting periods, 3·96 kcal ($P_{diff} < 0{\cdot}01$). This difference has a negligible effect on the energy balance because of the very small mass of the faeces during fasting, and is largely due to the increased nitrogen content of the faecal dry matter.

Urinary energy loss (Fig. 4). This, by its method of derivation, follows exactly the course of urinary nitrogen.

Energy balance. The total mean energy exchange with the daily apparent energy retention during the experiment is shown in Fig. 4. As total ingested energy was reduced to zero on the first day of fast, while energy expenditure and faecal energy loss did not reach their minima until the second day of deprivation, the negative apparent energy retention was greater on the first day than on the second. The gross mean loss of energy during the period of deprivation was 73·2 kcal. The gross mean energy gain during the recorded days of refeeding was 78·4 kcal.

Nitrogen exchange

Nitrogen intake (Fig. 5). During refeeding nitrogen intake increased correspondingly to the increased food intake. The nitrogen content of the food was 25 mg/g, corresponding to about 15 % of protein (estimated on food containing 10 % water).

Urinary nitrogen (Fig. 5). The mean daily pre-fasting level was 227 mg/day. This fell during the first day of fasting to 196 mg (86 %), with only a slight further fall to 187 mg during the second day of fasting. During the refeeding period the total daily urinary nitrogen, 234 mg, was only slightly (but significantly, $P < 0{\cdot}01$) higher than that during the pre-fasting period.

Faecal nitrogen (Fig. 5). The nitrogen content per gram of dried faeces during the non-fasting days was quite variable, but the mean, $29{\cdot}7 \pm 0{\cdot}7$ mg/g, approximated to that of dry food (27·5 mg/g).

During the fasting period the nitrogen content of faeces rose irregularly on the first day (35·9 mg/g) and consistently and markedly on the second day (49·6 mg/g). This increase affects the partitioning of increments of gut

contents and tissue quite appreciably, but does not notably affect the fall in total nitrogen loss by this route during fasting. During normal feeding the nitrogen of the faeces made up about one-third of the total nitrogen turnover: during fasting it made up about one-eighth.

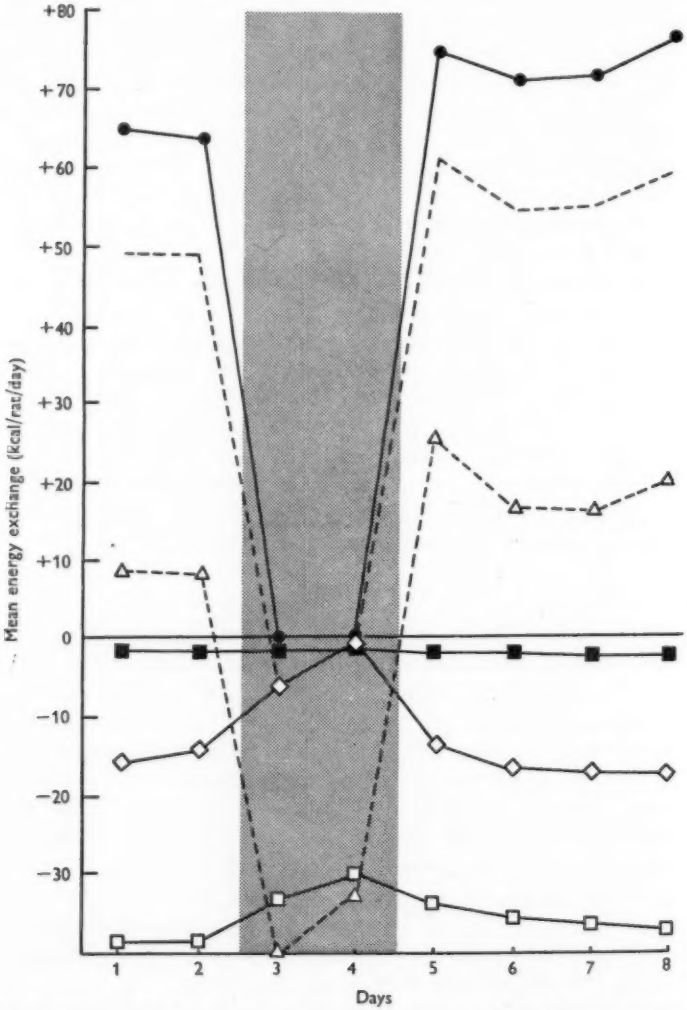

Fig. 4. Components of mean energy exchange: ● energy intake, ◇ energy of urine, ■ energy of faeces, □ energy expenditure, △ energy balance, - - - - - - absorbed energy intake.

Nitrogen balance. The components of nitrogen exchange are shown in Fig. 5. The mean apparent retention was positive during all the non-fasting days. In only one experimental period with one animal was there a loss of nitrogen (−29 mg) during the pre-fasting period. In two runs of another animal the balance was positive and large during the pre-fasting period, +326 and +336 mg for the 2 days. In the other five runs the balance varied from +1 to +89 mg.

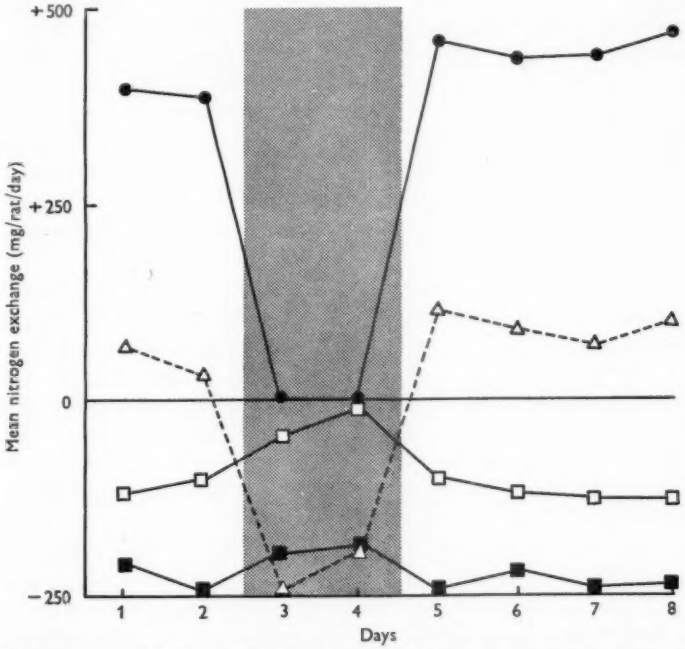

Fig. 5. Components of mean nitrogen exchange: ● total nitrogen in ingested food, ■ urinary nitrogen, □ faecal nitrogen, △ nitrogen balance.

The negative apparent retention on the first day of fasting was greater than on the second day. This, as with energy retention, was largely due to the greater total nitrogen loss in faeces during the first day.

During the refeeding period the gross apparent retention of nitrogen rose, on the first day of refeeding, to +111 mg and remained consistently higher than the pre-fasting period retention throughout the 4 days of refeeding ($P < 0{\cdot}01$). The balance was negative for the first day of refeeding in one run. All other refeeding balances were positive. The cumulative positive retention of nitrogen during the recorded period of refeeding (377 mg) was rather less than the loss during deprivation (436 mg) ($P = 0{\cdot}02$).

The increased positive apparent nitrogen retention during the refeeding period above that obtaining during the pre-fasting period was entirely due to the increased nitrogen intake during refeeding. There was no evidence of change in fraction of nitrogen absorbed nor of change in the rate of urinary loss with intake.

Water exchange

Water intake. The average pattern of intake shown in Fig. 6 was also true for individual runs during the pre-fasting and refeeding periods. During the period of deprivation, however, the fluid intake was so variable, both between animals and between runs on the same animal, that average values have little significance. In three instances the intake rose to over twice normal, and in three others fell to almost zero on the first day of fasting. Of the five other runs, two showed a rise of about 20 % and one a fall of 30 % in intake. The water intake on the second day of fasting was also very variable but without such extreme changes. The direction of changes in intake on the second day of fasting bore no consistent relationship to the direction of changes on the first day.

Food moisture (Fig. 6). The moisture content of the food was 10 % and food moisture contributed 1–2 g/day to the total water intake normally.

Metabolic water (Fig. 6). This contributed 4–6 g/day to the total water intake during normal feeding and 2–3 g/day during deprivation.

Urinary water loss (Fig. 6). During the pre-fasting period this loss was about 7 g/day, and during the refeeding period about 8 g/day. During fasting the mean urinary water was 18·3 g on the first day and 12·6 g on the second. (All these values, and those in Fig. 6, are for urine water and not for total urine.)

Although there was, again, some fluctuation in level between individual runs, the pattern was much more consistent in detail than it was for fluid intake. The three runs of the preliminary experiment all showed amounts of urine water during fasting 2–4 times as great as the amounts during the pre-fasting period. Of the eight runs in the main experimental series, five showed the average pattern; one showed no change in amount of urine on the first day and a rise on the second, and two showed falls to about half normal on both days. These three runs were those in which fluid intake dropped nearly to zero on the first day of fasting. Those runs in which the water intake showed a rise or moderate fall all showed the raised output of urine.

Vaporized water loss. The problems of obtaining a reliable value for this loss by the methods of indirect calorimetry have been discussed in an earlier paper (Morrison, 1956). The method described there for making adjustment for the addition of water evaporated from other sources (notably faeces) to physiologically evaporated water has been used here.

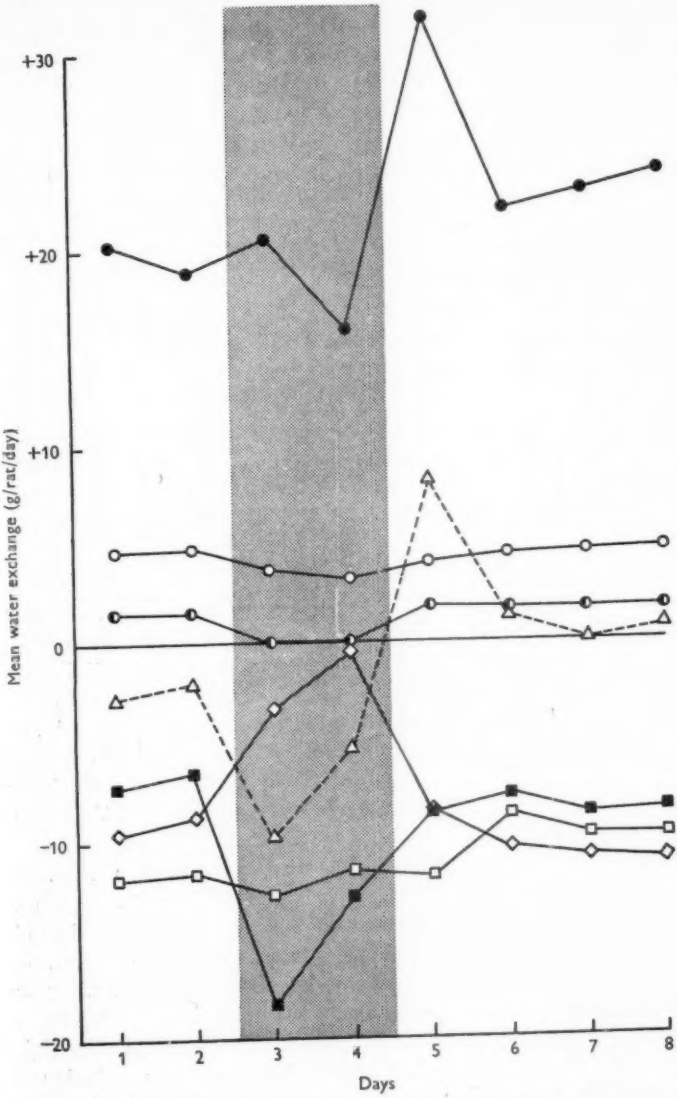

Fig. 6. Components of mean water exchange: ● fluid intake, ○ metabolic water, ◐ food moisture, ■ urinary water, ◇ faecal water, □ evaporated water, △ water balance.

The corrected values (Fig. 6) show that there was no substantial change in vaporized water during the fasting period. With the fall in mass of faeces during fasting, the values for vaporized water during that period are probably more accurate than at any other time. The fraction of the total heat eliminated by this route thus rises from about 18 % normally to about 23 % during fasting. It is probable that the apparent fall in evaporated water during the last 3 days of refeeding is an artifact arising from the block correction for evaporation of faecal water during the period of refeeding.

Water balance. Gross mean water retention during the pre-fasting period was slightly negative (Fig. 6). Only 4 individual values (out of 16) were positive. During food deprivation all individual values were negative; the mean values were 10 g lower on the first and 5 g lower on the second day of fasting than were the mean pre-fasting values. The mean values during refeeding were all positive, but 11 individual values (out of 32) were negative. Only on the first day of refeeding was the positive retention high (+8·3 g). Each refeeding day of each run had a water retention of 2 g or more greater than the pre-fasting values. The gross mean water lost during fasting was 15·3 g or 58·5 % of the body weight lost, while the water regained during refeeding was 10·7 g or 47·7 % of the body weight regained.

Partition of increments between body tissue and gut contents

The mean cumulative increments in dry matter, energy, nitrogen and water, along with the corresponding partitions of these into the parts attributable to increment in gut contents and the parts attributable to increment in body tissue, are shown in Fig. 7. For each variable considered the magnitudes of the calculated increments in tissue are reduced when the effect of increment in gut contents is removed. The difference is, of course, quantitatively much greater during the fasting period and at the beginning of the refeeding period, but even in the pre-fasting period the differences between gross and tissue increments are appreciable.

During the first day of refeeding the gross increments were, for dry matter, energy and water the largest daily increments of the whole refeeding period, and for nitrogen as large as any other. The tissue increments for all of these were, on the other hand, small on that day. For the subsequent days of refeeding the tissue increments in dry matter, energy and nitrogen were almost identical with the gross increments; the curves for the last 3 days of experiment are coincident or parallel. The high food intake on the first day of refeeding was thus largely a reflexion of gut filling; the high food intakes on subsequent days represent a true increase in nutrient absorption.

The increments in tissue water are completely different from the form suggested by the gross water increments. There was no loss of water from

tissue during the fasting period, and such loss as occurred over the whole experiment mainly occurred on the first day of the pre-fasting period.

Body composition

Average values for the water, fat and protein contents of male rats of initial body weight (excluding gut contents) comparable with those used

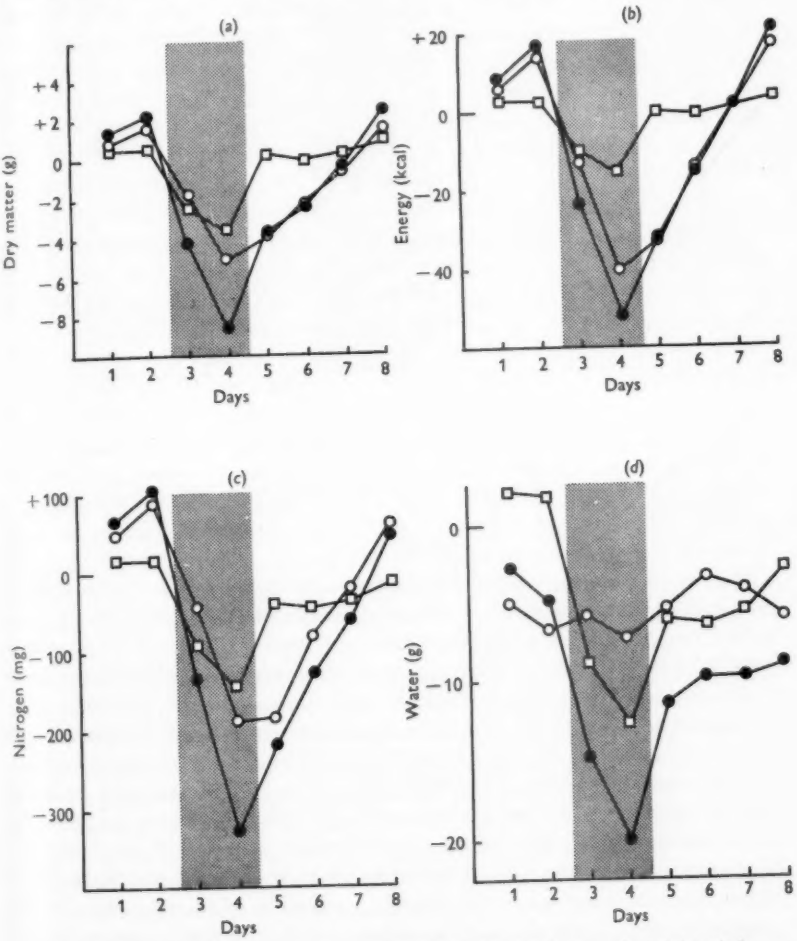

Fig. 7. Mean cumulative gross increments per rat during the experimental period, and their partition into increments in tissue and in gut contents. (*a*) Dry matter; (*b*) energy; (*c*) nitrogen; (*d*) water. In each, ● is total increment, ○ is tissue increment and □ is increment in gut contents.

in this experiment are 67, 9 and 19% respectively (Spray & Widdowson, 1950; Cumming & Morrison, unpublished work). With these figures as a starting point the change in body composition throughout the experimental period has been calculated by applying the tissue increments of water, protein and fat. The variations in tissue composition throughout the experimental period were slight (Fig. 8).

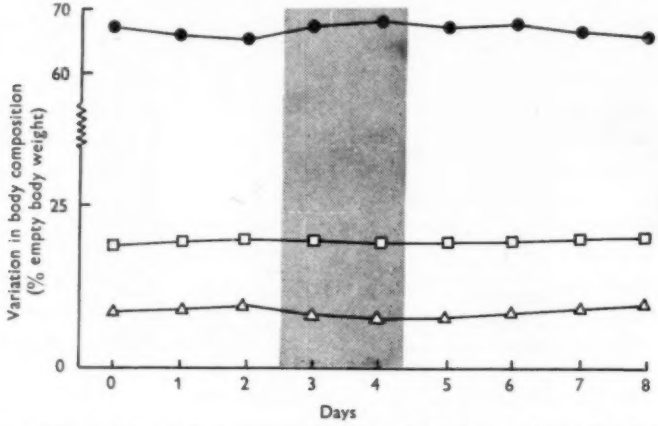

Fig. 8. The variation in body composition throughout the experimental period as percentage of body weight excluding gut contents: ● water, □ protein (N × 6·25), △ fat. The composition on day 0 was derived from estimations made on other comparable rats.

DISCUSSION

The first observation that the energy expenditure of a mammal falls during starvation was made by Regnault & Reiset (1849). This finding has been repeatedly confirmed. What has continued in doubt is whether the metabolic rate also falls. The number of attempts to answer this problem has been large, including studies on man, farm animals and laboratory animals. The variation in findings is illustrated by a series of reports from the same laboratory on rats. Benedict & MacLeod (1928) reported that expenditure per square metre of body surface fell in the first 24 hr of fasting and thereafter remained constant; Horst, Mendel & Benedict (1930) reported that expenditure by the whole animal fell continuously, that per unit of body weight it fell on the first or second day of fasting and thereafter remained constant and that per unit of body surface it fell gradually throughout the fast; and Benedict & Fox (1934) reported that expenditure fell markedly throughout the fast by any method of expression.

There are many reasons for this disagreement, but much of it has arisen through the use of short-term periods of metabolic study (seldom over 4 hr

and usually less) within the fasting period, and through the problem of a reference basis for the expression of metabolic rates. The almost invariable use of short-term periods of metabolic study has its origin partly in the technical inconvenience of longer periods and partly in the confining tyranny of the concept of 'basal' rates. Many of the investigations into the effect of fasting had, as their primary object, the establishment of the earliest or optimum post-absorptive time at which basal determinations could accurately be made (e.g. Benedict & MacLeod, 1928; Wesson, 1931). In very few investigations has the energy expenditure or the food intake been recorded for the pre-fasting period, and where these have been recorded (Giaja & Males, 1926; Wesson, 1931; Kingdon, Bunnell & Griffith, 1942) the food intake has not been taken into account in deriving the metabolic rate.

The problem of what unit of size to use as a standard of reference for metabolic rates has yet to find a satisfactory solution. It is sufficient to note here that a polynomial in W and W^2 does not fit the present data significantly better than the simple regression on W ($P > 0{\cdot}1$).

It is apparent from the present work that there was no change in total metabolic rate over either day of the 48-hr fast to which the rats were subjected; neither was there any change in rate during the refeeding period when both change in body weight and change in food intake (i.e. the total heat increment of feeding) were taken into account (Fig. 1*a* and Table 3). This is in agreement with the finding given in a preliminary report based on four experimental periods (Cumming & Morrison, 1955). When, however, the metabolic rate was based only on body weight the rate appeared to fall on the first day of fasting and to rise on the first day of refeeding. It seems likely that the fall in rate reported during the first day of fasting in all other studies, and also the elevated rate reported at the beginning of refeeding by Pembrey & Spriggs (1904), by Quimby, Phillips & White (1948) and by Blaxter & Graham (1955) resulted from failure to take into account the energy cost of feeding, of digestion and of metabolism. Kingdon *et al.* (1942) reported particularly a transient but large rise in heat production and R.Q. of rats 22–26 hr after the beginning of fasting. A transient rise in R.Q. is not very meaningful. It seems possible, from our continuous record of oxygen consumption, that the rise in heat production found by Kingdon *et al.* may have been due to the occurrence of a peak in the diurnal cycle (Fig. 3). In order to control precisely the time at which food deprivation started, these workers trained their rats to eat their entire food requirement for the day in two 2-hr periods. From their results an alternative possibility appears to be that the rise in metabolic rate which they observed was coincident with the expected but unfulfilled meal-times at the end of the first full day of fasting.

It is possible that the total M.R. might be sustained during the early period of fasting (which is all that has been studied here) by an increase in activity consequent on food deprivation. There is some evidence for a raised activity during fasting (Hitchcock, 1928). Benedict & MacLeod (1928), however, claimed that there was no increase in activity during fasting. The present work also suggests that there is no increase in activity, as such an increase would be expected to increase the amplitude of the diurnal variation in oxygen consumption, whereas in fact an insignificant fall occurred (Fig. 3).

The high diurnal variation during refeeding (Fig. 3) is probably partly accountable to the increased energy cost of feeding with the high food intakes which occurred during the refeeding period. The increasing amplitude of variation on successive days of refeeding would similarly be partly accountable to the effect of increasing body weight on the energy cost of feeding (other than specific dynamic action.). The increasing amplitude during refeeding may also represent some variable component of activity not directly related to feeding. If such an independent activity component were quantitatively important it could be expected to appear in the form of a high residual variance after removal of the multiple regression, and this residual is, in fact, very low (Table 3).

Another possible way in which M.R. might be held elevated is by a relative increase in more rapidly metabolizing tissue. The heat production of adipose tissue is low (Shapiro & Wertheimer, 1956) and if the relative amounts of fat, protein and water in the tissue were greatly altered during fasting and refeeding the aggregate tissue metabolic rate might appear to rise. The very slight changes in concentration of water, fat and protein of the tissue (Fig. 8) indicate that change in tissue composition is unlikely to have had an appreciable effect in the present experiments. The eventual rise in M.R. observed by Benedict, Horst & Mendel (1932) in prolonged fasting of initially very large rats might be explained by such an effect.

Although there is no change in the total concentration of water in the tissue there might well be a change in the ratio of intracellular to extracellular water. The intra- and extracellular fluid spaces have been found to be related to the energy expenditure (Dahlström, 1950; Robinson, 1950; Wedgewood, Bass, Klimas, Kleeman & Quinn, 1953). These spaces have not been measured in the present work. An implication of the part of the present work dealing with partition of water into tissue water and water of gut contents, and already suggested by Cizek (1954), is that the extracellular water as measured is not necessarily at all closely related to the extracellular water actually associated with the tissues.

Another aspect of the possible effect of varying proportion of tissue of

different metabolic rate is the changing amount of the absolutely non-metabolizing component of observed body weight, namely gut contents. It has been seen from the analysis of the results with body weight with gut contents held constant that the removal of the variation in gut contents unmasks a large change in metabolic rate in this experimental sequence of fasting and refeeding. The conclusion must be, therefore, that while the total metabolic rate of the intact normal animal does not alter during fasting and refeeding if changes in both body weight and food intake are taken into account, the metabolic rate of the tissues does fall even when the heat increment of feeding is taken into account.

The partition technique which has been employed to estimate the increments in tissue during the experimental period requires complete balance figures for body weight, energy, nitrogen and water. The calculation entails a number of assumptions which may impose an absolute error on the partition. But as the partition for each day on each animal is derived from and is therefore consistent with the measured 24-hr metabolic exchanges, the relative changes shown by this partition method are likely to give a truer picture for a given experiment on a given animal than would the application of average values obtained by slaughter. In recent years the studies of Dewar & Newton (1948) and Dewar (1953) on mice, of Morrison (1955, 1956) and Pratt & Putney (1958) on rats, and of Blaxter & Graham (1955), Graham, Wainman, Blaxter & Armstrong (1959) and Blaxter, Graham & Wainman (1959) on sheep have obtained all the necessary information. We can find no record, however, that this partition has ever been made.

The great differences shown in Fig. 7 between the gross increments and the calculated tissue increments during fasting and the beginning of refeeding demonstrate that attempts to describe and analyse tissue increments on the basis of gross observed balances, however accurately these may be done, are liable to be wildly in error wherever a steady state of gut contents is not known to exist. This applies not only to fasting experiments but also to overfeeding experiments, and to any situation where the mass of gut contents might alter. The appreciable change in calculated gut contents shown in the pre-fasting period (Table 1) demonstrates that it is difficult to assess from simple measurements when gut contents may be altering. The experimental rats had been on the same diet since weaning, and during the pre-fasting period body weight, food intake, water intake, urinary water, total heat production and non-protein R.Q. were all stable within normal limits.

The liability to serious errors arising in this way will obviously be greater with short periods of study. Some of the anomalous findings in reduction of obesity may possibly be explained in this way. Kekwick & Pawan (1956)

reported that obese patients lost body weight more rapidly on a diet in which 90% of the energy was derived from fat than on diets in which 90% of the energy was derived from either carbohydrate or protein. The diets were given iso-calorically (1000 kcal/day) and each study on each diet lasted 7 days. The nutrient component of the high fat diet had almost twice the caloric density, and, therefore, little more than half the bulk of either of the other two diets for iso-caloric amounts. As the additional weight lost on the high fat diet above that lost on the high protein or high carbohydrate diets averaged less than 1 kg in the entire period of study, certainly some and possibly most of this could be accounted for by change in gut contents. Fully absorbable carbohydrate or protein diets given to 100 g rats produced gut contents about 1 g greater than that found with a fat diet, when the diets were given iso-calorically (Miller & Kriss, 1934).

Apart from the differences in magnitude between tissue and gross increments shown for dry matter, energy and nitrogen (Fig. 7), the most striking feature of the tissue increments is the time lag between beginning refeeding and the beginning of appreciable tissue gain. Almost a complete day was required before the complex of gut filling, transport and absorption was able to meet, equal and overcome the endogenous drain imposed by fasting. Ivy (1958) and Ju & Nasset (1959) have shown that, of the gastro-intestinal tract, only the major directly absorbing part, the small intestine, gained weight and nitrogen on the first day of refeeding after a 48-hr fast.

The partition of water gain between tissue and gut contents has already been stated (Methods) to be much less reliable than the partition of dry matter. The remarkable change in form of increment between total and tissue increment (Fig. 7*d*) is, we believe, too great to be attributed only to error in partitioning. The part at which error is most likely is in the last 2 days of refeeding where the hydration of gut contents may be lower than has been estimated. Such an error would lead to a smaller decrement in tissue fluid than has been estimated, i.e. would make the change in form even more pronounced. The hydration of gut contents found here for normal feeding and fasting is in close agreement with other work (Aach, Rolf & White, 1957; Cizek, 1954). The only published results on refeeding after fasting (Lepkovsky, Lyman, Fleming, Nagumo & Dimick, 1957) gave lower values for hydration of gut contents than are used here, but the conditions of experiment are not closely comparable.

The tissue water remained fairly constant throughout the experimental period, apart from a marked fall during the first day of the pre-fasting period. The mean total water loss during the fasting period, found from the gross water increments, was 15·3 g, while that from the estimated tissue decrement was 0·7 g. This result is in contrast to that

given in a preliminary report by Cumming & Morrison (1956), in which no account was taken of changes in gut contents.

Again, these differences between gross water exchange and tissue water exchange point the danger of embarking on elaborate physiological interpretations of water increments derived by any of the variants of equation (3). To use this equation to derive water increment involves a double error, the assumption that all non-protein dry-matter increment is tissue increment and is fat, and the assumption that all water increment is tissue-water increment.

The progressive slight dehydration of tissue during the pre-fasting period is presumably attributable to the existence of the animal in the indirect calorimeter. We do not know, at present, why this should occur.

The physiological origins of the variations in water intake and, particularly, the greatly elevated urine volumes during the fasting period have been made the subject of further work presently in preparation.

SUMMARY

1. All the components of the energy, nitrogen and water exchanges of five adult male rats have been recorded continuously for a total of 11 experimental periods of 5–8 days. Each experimental period consisted of a pre-fasting period of 2 days, 2 days of starvation and several days of subsequent refeeding.

2. A method was elaborated by which the total observed increments of dry matter, of nitrogen, of energy and of water could be partitioned into the parts attributable to increment in gut contents and the parts attributable to increment in body tissues.

3. The total energy expenditure of the rats fell during the 48-hr fast and rose during refeeding.

4. When the changes in body weight and in food intake were allowed for, the metabolic rate was found to remain constant throughout the whole experimental period.

5. When the effect of changes in mass of gut contents was eliminated, the metabolic rate was found to fall during the period even when changes in food intake were taken into account.

6. The gross increments in dry matter, in energy and in nitrogen during fasting and refeeding were much greater than the calculated tissue increments. On the first day of refeeding most of the gross increment in dry matter could be attributed to refilling of the gut. Only a small amount of tissue was regained on the first day of refeeding.

7. The variations in gross water increment give a very misleading conception of changes in tissue water increment. No appreciable change in tissue water occurred during the fasting period.

8. Balance studies in underfeeding or overfeeding situations, where the methods do not allow for estimation of variation in gut contents, are liable to serious errors.

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THE CATECHOLAMINE CONTENT OF THE PERIPHERAL PLASMA IN HUMAN SUBJECTS WITH COMPLETE TRANSVERSE LESIONS OF THE SPINAL CORD

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It is now believed that noradrenaline is the main and possibly the only catecholamine liberated at post-ganglionic adrenergic nerve ending (Peart, 1949; von Euler, 1951). There is still doubt however whether, in the intact animal, any of this noradrenaline finds its way into the circulation (Celander, 1954). Using sensitive chemical methods various workers (Weil-Malherbe & Bone, 1953; Valk & Price, 1956; Cohen & Goldenberg, 1957; Robinson & Stott, 1958; Munro & Robinson 1958*a*; Keenan, Kleitsch & Humoller, 1959), have consistently found noradrenaline, as well as adrenaline, in the normal human plasma although the levels observed have varied widely. The origin may be, of course, the adrenal medulla rather than the sympathetic nerve endings, but the information available on this point from animal experiments is difficult to interpret. If, as appears likely, adrenaline and noradrenaline are liberated into the peripheral blood stream, the question arises whether the concentration present at a particular time reflects the prevailing level of activity of the sympathetic and adrenal-medullary systems.

Our particular interest was the sympathetic nervous system in human spinal subjects; and with this object measurements of plasma adrenaline and noradrenaline were made on a group with complete transverse lesions at different levels of the spinal cord. If noradrenaline is liberated into the blood in proportion to the degree of sympathetic nervous activity, it might be expected that the circulating level in resting spinal subjects would be below the normal and possibly related to the level of the cord lesion. Furthermore, some information might be gained regarding the character of the human adrenal-medullary secretion by noting the change, if any, in the proportion of plasma adrenaline to noradrenaline occurring within the range of lesions at the spinal levels giving origin to the nerves

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supplying the gland. For comparison, data have been included for a group of normal persons and another group before and after bilateral adrenalectomy.

Eosinophil counts were made on blood samples from the spinal group. Since normal individuals develop an eosinopenia in response to injected adrenaline, it appeared likely that a significant eosinophilia in the spinal group could be taken as additional, although admittedly indirect, evidence of subnormal levels of adrenaline in the plasma.

Chemical methods of assay for adrenaline and noradrenaline in peripheral plasma have been criticized on the grounds of specificity. Biological methods have been regarded as more acceptable in this respect, but they lack the required sensitivity. We have used in the present investigation the chemical method originally developed by von Euler & Floding (1955*a*, *b*) for analysis of adrenaline and noradrenaline. The method has been modified by the incorporation of extra blanks and by using a highly sensitive fluorimeter (Robinson & Stott, 1958).

METHODS

The blood samples were collected from the median cubital vein into 20 ml. all-glass syringes containing the anticoagulant. Unless otherwise stated the blood was collected after the subjects had been seated for 2–3 min but not specially rested.

Blood samples were obtained from sixteen healthy medical students and laboratory workers. Since posture affects the catecholamine values (Munro & Robinson, 1958*b*) the collections were made from these subjects in both the supine and the upright sitting positions so that the results could be more strictly compared with those of other workers.

Blood was also collected from forty patients with complete transverse lesions of the spinal cord, the result of fracture-dislocations of the spine. The level of the cord lesions varied from the 4th cervical to the 3rd lumbar segments. These patients were in the upright sitting position during blood sampling and were well recovered from their initial injury.

Blood samples were also obtained from eight female patients before and after bilateral adrenalectomy for carcinoma. The first samples were collected 2–4 days before the first operation and the second 6–8 days after the second operation. The collections in these cases were made with the patients lying in bed. A ninth patient was examined 9 months after operation for bilateral adrenalectomy but not before operation.

Five millilitres of a solution containing sodium fluoride 2 g and sodium thiosulphate 3 g/100 ml. were drawn into a 20 ml. syringe, and the blood sample then drawn from the medial cubital vein up to the 20 ml. mark. With the minimum of delay the blood was centrifuged at approximately 2000 rev/min and the plasma separated.

In a few of the normal subjects blood was collected with special precautions to maintain the integrity of the plasma. The collection was made into silliconed syringes containing approximately 500 i.u. heparin in a volume of 0·05 ml. Immediately after collection it was put into siliconed centrifuge tubes packed in ice and centrifuged as above, and the plasma separated (Holzbauer & Vogt, 1954).

Eosinophil counts were carried out by the method of Pilot (1950) on forty-five fit subjects with spinal cord lesions of long standing. These subjects were in the upright sitting position during sampling.

The catecholamines were isolated on micro-columns of alumina by the method of

Weil-Malherbe & Bone (1952). The adrenaline and noradrenaline content of the eluate was determined fluorimetrically by a modification (Robinson & Stott, 1958) of the method of von Euler & Floding (1955*a*, *b*).

RESULTS

Adrenaline and noradrenaline levels in peripheral plasma

Normal subjects. In normal healthy persons who had been sitting upright for 2–3 min after being engaged in the usual laboratory and ward duties the plasma adrenaline, as shown on Table 1, was $2{\cdot}3 \pm 0{\cdot}36\,\mu g/l.$ (mean of sixteen subjects $\pm$ S.D.) and the noradrenaline $4{\cdot}28 \pm 0{\cdot}64\,\mu g/l.$ (mean of fourteen subjects).

After the subjects had been supine for 40–50 min the corresponding values were: adrenaline $1{\cdot}72 \pm 0{\cdot}51\,\mu g/l.$ (mean of seventeen subjects); and noradrenaline $2{\cdot}2 \pm 0{\cdot}26\,\mu g/l.$ (mean of seventeen subjects). The differences between the adrenaline values of the subjects in the supine and upright sitting positions is not statistically significant but the difference between the noradrenaline figures is significant ($P < 0{\cdot}05$).

Samples taken as described by Holzbauer & Vogt (1954), i.e. into heparin in siliconed syringes from four normal seated subjects, gave adrenaline and noradrenaline values very much lower than those quoted above. The mean adrenaline value as shown in Table 1 was below the threshold of measurement by the method used, i.e. $< 0{\cdot}3\,\mu g/l.$ plasma, whilst the mean for noradrenaline was $0{\cdot}9\,\mu g/l.$ (range $0{\cdot}6$–$1{\cdot}6\,\mu g/l.$) The difference between the results when the blood is taken into the sodium fluoride and sodium thiosulphate solution and when taken into heparin is altogether outside the range of experimental error or of individual variation.

Patients with complete transverse lesions of the spinal cord. Figure 1 shows the mean values for plasma adrenaline and noradrenaline, plotted in the form of histograms, in relation to the segmental level of the cord lesion. Plasma values for adrenaline and noradrenaline of normal subjects are also shown for comparison. There are considerable differences between the plasma adrenaline and noradrenaline values of the normal and of the spinal subjects with lesions high in the cord.

Table 2 shows that the concentration of plasma adrenaline in patients with complete cervical lesions is just above the limit of measurability with the methods used ($0{\cdot}3\,\mu g/l.$). These low values persist in cases with lesions down to a level below T3. A distinct increase occurs with lesions below T4 and T5 but the values are still below those found in normal controls. The plasma adrenaline attains its maximum in subjects with lesions below T6 and from this point is not significantly different from the normal value.

The changes in plasma noradrenaline are comparable to those of

TABLE 1. Comparison of reported values for adrenaline and noradrenaline in the peripheral plasma of normal human subjects

Condition of subjects	Method of assay*	Adrenaline (μg./l. $\pm$ S.D.)	Noradrenaline (μg./l. $\pm$ S.D.)	Method of collecting blood*	Authors
Seated for 3 min after normal activity	THI	2·3 $\pm$0·36	4·28 $\pm$0·64	Fluoride-thiosulphate	Munro & Robinson (1958*a*)
After lying supine for 50 min	THI	1·72 $\pm$0·51	2·2 $\pm$0·26	Fluoride-thiosulphate	Munro & Robinson (1958*b*)
After lying down for 30 min	EDA	1·3	4·1	Fluoride-thiosulphate	Weil-Malherbe & Bone (1953)
Seated for 3 min after normal activity	THI	<0·3	0·9	Siliconed heparinized syringe	Munro & Robinson (1958*b*)
	THI	0·00 $\pm$0·01	0·2 $\pm$0·19	Heparin	Valk & Price (1956)
	EDA	0·097 $\pm$0·14	—	Heparin	Millar (1956)
	THI	0·06 $\pm$0·05	0·3 $\pm$0·07	Heparin	Cohen & Goldenberg (1957)
	Rat uterus	0·06	<1·0	Siliconed heparinized syringe	Holzbauer & Vogt (1954)

* THI indicates a trihydroxyindole method; EDA indicates an ethylene diamine condensation method. The blood samples were collected into solutions of the substances indicated.

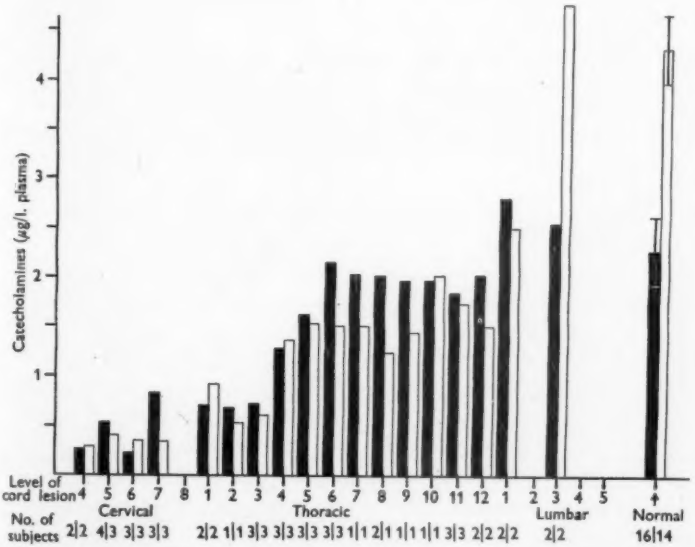
Catecholamines (μg/l. plasma)
4
3
2
1
Level of cord lesion
4 5 6 7 8 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5
Cervical
Thoracic
Lumbar
Normal
No. of subjects
2|2 4|3 3|3 3|3 2|2 1|1 3|3 3|3 3|3 3|1 1|2 1|1 1|1 1|3 3|2 2|2 2|2 16|14

Fig. 1. Plasma adrenaline (■) and noradrenaline (□) in patients with spinal lesions at different levels, and in normal man.

adrenaline in so far as very low values are found with cervical and high thoracic cord lesions. A relatively abrupt rise in concentration of plasma noradrenaline also occurs with a level of lesion just below T3, but again the values obtained with lesions below T4 and T5 are definitely lagging behind those of the normal controls.

The noradrenaline values rise gradually with lowering of the level of lesion down to L1 and then rise abruptly between L1 and L3 to the normal value. Table 2 also shows the statistical significance to be attached to the above data.

TABLE 2. Plasma adrenaline and noradrenaline concentrations in subjects with complete transverse lesions of the spinal cord at different levels

Level of cord lesion	Adrenaline (μg/l. $\pm$ S.D.)	Noradrenaline (μg/l. $\pm$ S.D.)	
C4–T3	0·4 $\pm$0·13	0·25 $\pm$0·24	15
T4–T8	1·65 $\pm$0·48	1·15 $\pm$0·49	9
T9–L1	1·92 $\pm$0·62	2·0 $\pm$0·47	9
L3	2·0 $\pm$0·70	4·6 $\pm$1·2	2
Normal	2·33 $\pm$0·47	4·1 $\pm$0·46	16

Significance of differences (t test)

Between	Catecholamine determined	P
C4–T3 and T4–T8	Adrenaline	$<0·05$
C4–T3 and T4–T8	Noradrenaline	$<0·01$
T9–L1 and normal	Noradrenaline	$<0·01$
L3 and normal	Noradrenaline	Not significant

The ratio of noradrenaline to adrenaline also varies. In normal subjects the plasma noradrenaline is always the higher, but with few exceptions the reverse occurs in the spinal subjects at all segmental levels between T4 and L1. On the other hand, in those with lesions at L3 the proportion is the same as in normal subjects. It would appear that some change of physiological significance, probably related to sympathetic nerve activity, occurs in subjects with lesions between L1 and L3.

Subjects before and after bilateral adrenalectomy. In this group blood samples were taken with the patients lying in the supine position. Before operation the plasma adrenaline did not differ significantly in value from that of normal subjects in the supine position. It was $1·75 \pm 0·51$ μg/l. (mean of eight subjects). The second blood samples taken 6–8 days after the second stage of adrenalectomy showed, in eight out of the nine patients, plasma adrenaline values too low to be measured by the method used, i.e. less than 0·3 μg/l. The ninth patient had a plasma adrenaline of only 0·6 μg/l.

The plasma noradrenaline before the operation was $1·94 \pm 0·39$ μg/l.

(mean of eight subjects). After bilateral adrenalectomy the noradrenaline value rose to $2{\cdot}98 \pm 0{\cdot}76\,\mu g/l$. (mean of nine subjects). Of the eight patients who were studied both before and after adrenalectomy six showed a post-adrenalectomy increase in plasma noradrenaline, one a slight fall, and one no change. The differences between pre- and post-adrenalectomy values, however, are not statistically significant.

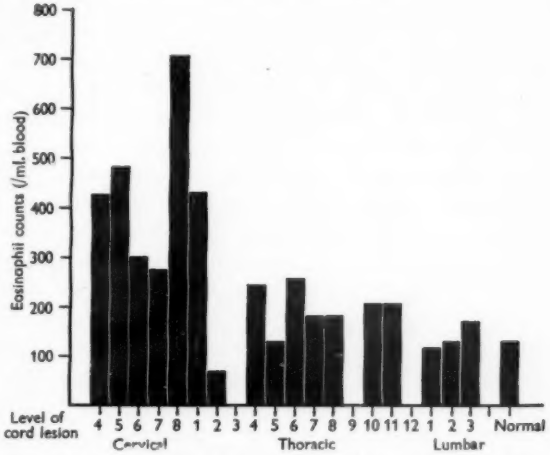

Fig. 2. Eosinophil counts in 45 patients with spinal lesions at different levels, and in normal man.

Eosinophil counts in spinal subjects

Figure 2 shows in histogram form the eosinophil counts of forty-five patients with complete and long-standing transverse lesions of the cord. Thirteen out of twenty-one patients with lesions above T5 showed some degree of eosinophilia, i.e. counts above 300/mm^3. Twenty-three patients with lesions complete at T5 and below all showed normal counts. The impression obtained is that eosinophil counts are about normal with lesions up to T2 level. With lesions immediately higher in the cord than this the eosonophil count rises significantly to an abnormal value and remains elevated about the same amount with higher levels of lesion. It is interesting to note the complementary character of the histograms for plasma adrenaline and eosinophil count.

DISCUSSION

Much of the previous work on the catecholamine content of the peripheral plasma has been concerned with the circulating levels of adrenaline and noradrenaline in the resting normal subject (Table 1), and the present

observations in this respect compare most closely with those of Weil-Malherbe & Bone (1953). These have been criticized, and their relatively high values explained, on the basis of a poor specificity of the method employed, but this cannot account for the present values, which were obtained by a different and more specific method of assay. The differences shown in the table appear to be due less to the method of assay than to the way in which the blood samples were taken. Weil-Malherbe & Bone (1958) found that human platelets contain adrenaline and noradrenaline and that they would take up both adrenaline and noradrenaline against a concentration gradient. More recently Born & Hornykiewicz (1957) and Born, Hornykiewicz & Stafford (1958) have made a similar finding with pig platelets. It is possible that the platelets, by their capacity to absorb catecholamines, form a 'trap' taking up any transmitter which escapes into the blood from the nerve endings and receptor tissue: this could serve to help to localize a sympathetic response. Inspection of Table 1 suggests that where great care is taken to maintain the platelets and the integrity of the plasma generally (Valk & Price, 1956), the adrenaline and noradrenaline values are at their lowest. Where maximum disruption of platelets is likely to occur, as in the fluoride solution, maximum catecholamine values are found. If it were possible to sample the completely undisturbed plasma then zero values might conceivably be found. On the other hand, in acute stress with maximum stimulation of the sympathetic and adrenal medullary systems the platelet trap might not be completely effective and measurable amounts of the catecholamines might be found 'free in the plasma'.

The present investigation shows that by measuring the 'total extractable' adrenaline and noradrenaline rather than the 'free' and biologically active, a reasonable estimate may be made of the prevailing level of sympathetic and adrenal medullary activity. For instance, the increase in plasma noradrenaline with an unchanged level of adrenaline when normal subjects rise from the supine to the sitting position suggests increased activity of the vasoconstrictor nerves associated with an escape of noradrenaline from the endings into the peripheral blood. Celander (1954), on the other hand, using a biological method of assay, was unable to demonstrate the presence of circulating sympathin even after maximal stimulation of the splanchnic nerves, that is, at a rate much higher than 6–8/sec, which Folkow & Uvnäs (1948) estimated as the upper limit of the physiological rate of discharge of sympathetic vasomotor nerves. Obviously the fall in the catecholamine content of the plasma with progressively higher levels of cord lesions reflects the increasing degree of isolation of the sympathetic chain from the normal tonic control by centres in the brain. Small amounts of adrenaline and noradrenaline nevertheless persist

in the peripheral plasma of patients with long-standing cervical lesions. Sympathetic reflex activity at the spinal level, the presence of extra-medullary chromaffine tissue, or direct stimulation of the adrenal medulla by circulating metabolites, may account for this. We have found for instance that the occurrence of active infection in spinal patients leads to higher than normal plasma adrenaline values.

Our observations confirm the view that the adrenal medulla is normally maintained in an active state by higher nervous centres. The plasma adrenaline values are significantly lower in the case of lesions above than below T4, but not different from the normal in the patients with complete lesions below T6. The main outflow of secretory fibres to the adrenal medulla thus seems to occur between T4 and T6. Teitelbaum (1942) on the basis of anatomical studies describes a much wider secretory outflow from T5 to T11. Vogt (1952) found that the denervated adrenal medulla of the cat continued to secrete both adrenaline and noradrenaline, but much less actively than the innervated gland. An abnormally high circulation of metabolites due to the operative procedures might at least partly account for the observed levels in the denervated animals.

The existing information with regard to the catecholamine composition of the adrenal medullary secretion has been mainly obtained by analysing the adrenal vein effluent in anaesthetized animals, i.e. under some degree of stress (Folkow & Uvnäs 1948; Vogt, 1952; Dunér, 1953). This indicates a significantly greater proportion of noradrenaline than adrenaline, which becomes more pronounced when the nerves to the gland are stimulated. The present investigation affords some information on this point in the unstressed human subject. Since spinal subjects with lesions below T4 have a predominance of adrenaline in the plasma, it may be assumed that a normal level of adrenal medullary activity which is apparent only in subjects with lesions below this level in the cord provokes a secretion composed predominantly of adrenaline.

The disappearance of adrenaline from the peripheral plasma after bilateral adrenalectomy suggests that all of it originates from the adrenal medulla and that the plasma adrenaline level in the intact subject is an index of the prevailing activity of this organ. The noradrenaline content of the plasma rose in most cases after bilateral adrenalectomy; it is unlikely therefore that this substance forms any considerable part of the medullary secretion in man. Von Euler, Franksson & Hellström (1954) observed a similar increase in urinary noradrenaline associated with a reduction of urinary adrenaline to 1/5 of the pre-operative level after bilateral adrenalectomy in human subjects. Since adrenaline has an important role in metabolism it may be that noradrenaline acting similarly but less strongly is increased in the circumstances to offset the loss of

adrenaline. The noradrenaline increase may also signify an enhanced stimulus to vasoconstriction in order to compensate for the loss of adrenocortical function.

It is difficult to provide any explanation other than a change in plasma catecholamine concentration which would fit the fact that eosinophilia occurs only in those patients with lesions sufficiently high in the spinal cord to isolate completely the splanchnic outflow from the higher nervous centres. There is so far no evidence that other substances capable of changing the eosinophil levels, e.g. histamine, undergo a change in concentration about this level of lesion. The possibility remains that the effect is only indirectly mediated by adrenaline, since it has been shown that the ketogenic steroid excretion of spinal patients follows approximately the same pattern when related to the level of the cord lesion as does plasma adrenaline (Robinson & Munro, 1958). That the adrenal cortical hormones are not necessary for an eosinopenic response to adrenaline is shown by the fact that it can be produced by adrenaline after removal of both adrenals. The present findings therefore suggest that subnormal levels of circulating adrenaline are directly responsible for the observed eosinophilia.

SUMMARY

1. The peripheral plasma levels of adrenaline and noradrenaline have been measured in a group of normal human subjects, in a group with complete transverse lesions of the spinal cord, and in another group before and after bilateral adrenalectomy. Eosinophil counts were made in a corresponding spinal group.

2. The plasma noradrenaline of the normal subjects was significantly higher in the sitting than in the supine position.

3. Both the adrenaline and the noradrenaline of the plasma were significantly below normal in spinal subjects with cervical and high thoracic lesions. The data provide evidence for the presence, normally, of tonic activity in the sympathetic nervous system dependent upon its functional continuity with higher nervous centres. Adrenal medullary activity is similarly affected.

4. The eosinophil counts in the spinal group were inversely related to the plasma adrenaline levels.

5. After bilateral adrenalectomy there was a significant reduction in plasma adrenaline but no corresponding fall in plasma noradrenaline.

We are most grateful to Dr L. Guttmann, the Director of the National Spinal Injuries Centre, for his interest and help during the course of this investigation, and in providing laboratory facilities for one of us (A.F.M). We wish to thank the Medical Research Council for an expenses grant to A.F.M. in connexion with this work, and the patients at the National Spinal Injuries Centre who co-operated as subjects in this investigation.

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THE OSMOTIC EFFECTS OF SOME SIMPLE MOLECULES AND IONS ON GASTRIC EMPTYING

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The idea that the rate of emptying of the stomach is strongly influenced by the osmotic pressure of the gastric contents stems from the experiments of Carnot & Chassevant (1905). They found that in dogs with duodenal fistulae saline nearly isosmotic with plasma left the stomach more rapidly than saline solutions which were more concentrated or more dilute. The influence of the concentration of salt on gastric emptying appears to be much the same in man as it is in dogs. Shay & Gershon-Cohen (1934) in their radiographic studies noted that tap water left the stomach more slowly than isotonic saline. They also concluded that in order to slow emptying—presumably relative to the emptying of tap water—'hypertonic' solutions of salts and glucose were necessary, whereas hydrochloric acid slowed emptying in solutions less concentrated than plasma in terms of milli-osmoles per litre.

The present experiments, extending those described by Hunt in 1956, are a study of the working of the receptors concerned in the slowing of gastric emptying that appear to be sensitive to the osmotic properties of the luminal contents of the duodenum and small intestine (Shay & Gershon-Cohen, 1934). The results of some experiments with acids which probably work differently (Sircus, 1958) are also described for comparison.

METHODS

After washing out the stomach with 250 ml. of tap water at about 8 a.m., test meals of 750 ml. of distilled water at 37° C containing various concentrations of solutes were given to four fasting volunteer medical students and the experimenters. The test meals contained, in addition to the solutes introduced as experimental variables, phenol red about 60 mg/l., which served as a marker and had no material influence on gastric emptying (Hunt, 1956). The meal was given down a tube into the stomach in about 75 sec. The tube was usually left down until the gastric contents were recovered some 10–30 min later, and the stomach was then washed out with 250 ml. tap water. The amount of phenol red recovered in this wash-out divided by the concentration of phenol red in the previously recovered major portion of gastric contents gave the volume of gastric contents not aspirated previously, usually about 20 ml. The volume of the original meal remaining in the stomach at the time of the recovery was calculated from the amount of phenol red recovered. In arranging these

experiments the interval between giving the meal and recovering it was usually adjusted for each subject so that the volume of meal recovered was at least 100 ml. out of the original 750 ml. given. Strict adherence to the routine procedure was necessary to obtain reproducibility.

The data reported here were collected during five years: because of a realization that the data initially obtained were inadequate in range or number for a new purpose, an interval of years separates some of the experiments within a single series in the same subject. Although the subjects studied are fewer than we would wish, the large number of meals given, over a thousand, and their scatter in time do provide some safeguard against the results being relevant only to special circumstances. The intensive study of a few subjects was necessary to reduce the variability due to differences between subjects, so allowing quantitative comparisons of the action of solutes and mixtures of solutes. In some subjects who gave very variable results the experiments were ended shortly and these results are not considered.

RESULTS

The results record the relations between the volume of a test meal recovered from the stomach at a fixed time after its instillation and the milliosmolar (m-osm) concentration of solutes in the instilled meal. For the most part these relationships were linear, so that straight lines could be fitted to the data by the method of least squares. Where the relationship was not linear over the whole range, for example in Fig. 4, the lines were calculated only for that part of the range which appeared on inspection to be linear. Such lines are shown as full; curved lines, shown interrupted are fitted by eye.

The influence of some monovalent and divalent anions and cations on the volume of meal remaining in the stomach after a fixed interval

Figure 1 shows the relationship between the concentration of either hydrochloric acid or potassium chloride and the volume of test meal recovered after 20 min in subject J.N.H. It may be seen that hydrochloric acid was about three times more effective per osmole than potassium chloride in increasing the volume of test meal recovered from the stomach. Similar data for other electrolytes are given in Table 1. As the concentrations were raised, so the amount of the original test meal recovered from the stomach after a fixed interval increased. Hydrochloric acid was the most effective per osmole, ammonium chloride the least effective.

In previous work (Hunt, 1956; Pathak, 1959) it was noted that the threshold concentration for the slowing of gastric emptying by hydrochloric acid was about 20 m-osm, and in the present work that for potassium chloride seemed to be at least 40 m-osm. Accordingly the regression equations shown in Table 1 for solutions of hydrochloric acid and for solutions of potassium chloride include only data for test meals containing concentrations of acid of 20 m-osm or more, and concentrations of potassium chloride of 40 m-osm or more. Reference to the intercepts (a) of the regression lines for meals containing hydrochloric acid or potassium

chloride and the axis for volume, in part 1 of Table 1, shows that in W.D.L.P. and J.N.H. the mean values for the volume of meals of water remaining at 20 min are less than those shown in part 2. This finding is consistent with the view that there is a threshold concentration for the

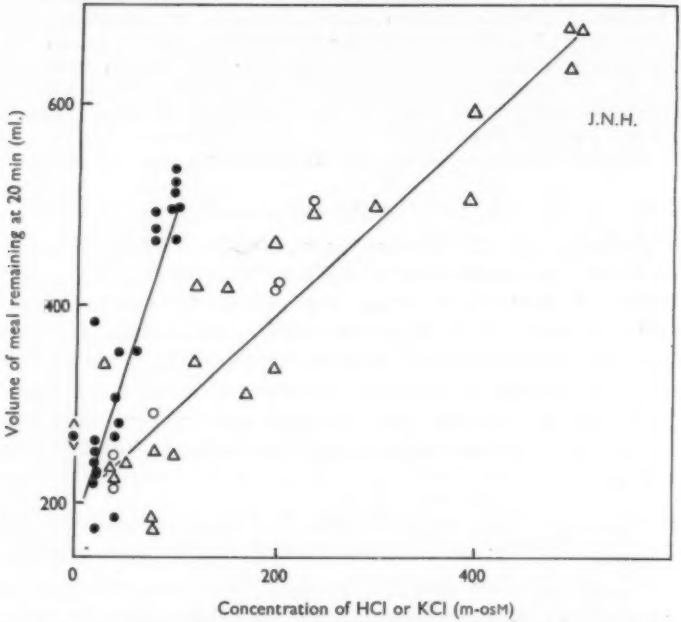

Fig. 1. A comparison of the influence of HCl (●) and of KCl (△) on the volume of test meals remaining in the stomach after 20 min.. ○ = KCl + 40 m-osmoles HCl.

slowing action of hydrochloric acid and of potassium chloride. It may also be seen in Fig. 1 that potassiun chloride was equally effective in slowing gastric emptying with and without 40 m-osM hydrochloric acid.

Table 1 also shows data for solutions containing ammonium chloride, which was less effective per osmole in slowing gastric emptying than either ammonium sulphate or calcium chloride. Putting these data together the conclusion seems warranted that calcium and sulphate were effective, whereas ammonium and chloride were relatively ineffective, in slowing emptying.

The influence of sodium salts on the volume of meal recovered after a fixed interval

All the solutes so far considered except ammonium chloride increased the volume of meal recovered as the concentration of solute in the meal

was raised. Sodium salts had a different action, as may be seen from Figs. 2 and 4 for example. As the concentrations of sodium chloride, sulphate or bicarbonate (Hunt, 1956) were raised, the volumes recovered from the stomach after a fixed interval of time fell until the concentration in the meal at the time of instillation reached about 250 m-osm with the chloride or bicarbonate, and about 50 m-osm with the sulphate. Data similar to those shown in Fig. 2 for J.N.H. were obtained for J.D.P. At higher concentrations all these salts of sodium increased the volume of the gastric contents recovered as the concentration in the test meal was raised.

TABLE 1. The influence of solutions of hydrochloric acid, sulphuric acid, potassium chloride, ammonium chloride, ammonium sulphate and calcium chloride on the volume of test meals remaining in the stomach after twenty minutes

Subject ...	J.D.P.				W.D.L.P.				J.N.H.			
Range of concentrations (m-osm)	n*	a	b	S.E. of b	n	a	b	S.E. of b	n	a	b	S.E. of b
HCl 20–100	14	375	2·66	±0·67	13	163	4·17	±0·65	22	180	3·07	±0·38
KCl 40–500	16	295	1·25	±0·20	30	265	0·95	±0·13	22	200	0·93	±0·25
	Mean volumes (ml.) of water remaining at 20 min											
Water	10	252 (S.E. ±18)			11	319 (S.E. ±15·3)			19	280 (S.E. ±12·5)		
	Regression lines fitted to data including those for meals of water											
NH_4Cl 0–200	19	260	0·44	±0·16	21	323	0·20	±0·14	26	279	0·005	±0·18
$(NH_4)_2SO_4$ 0–300	19	230	0·91	±0·13	15	318	0·90	±0·18	23	273	0·64	±0·18
$CaCl_2$ 0–300	32	295	0·81	±0·14	18	327	0·86	±0·14	35	260	0·44	±0·14
H_2SO_4 0–100	14	264	4·80	±0·84	14	321	2·89	±0·50	23	242	2·60	±0·37

* y = volume of meal remaining (ml.) at 20 min; x = concentration of solute (m-osm); n = number observations; a corresponds to the volume of meal remaining with zero concentration of solute, that the intercept of the regression line and the ordinate; b corresponds to the slopes of the lines as shown Fig. 1; and $y = a + bx$.

The influence of glucose and sodium chloride together on the volume of test meal recovered after a fixed interval

Glucose added to test meals increased the volume of the test meal recovered as the concentration was raised (Hunt, 1956) like the solutes referred to in Table 1. Figure 3 gives data on the effect of various concentrations of glucose, on the relation between the concentration of sodium chloride and the volume of meal recovered after 30 min. It may be seen:

(1) That 382 m-osm glucose in the meal apparently made the volume recovered independent of the concentration of sodium chloride in the meal, that is, the highest line on the figure is horizontal;

(2) That increase in the concentration of glucose increased the volume recovered from the stomach in the presence of various concentrations of sodium chloride;

(3) That the minimum volume recovered occurred with about 50 m-osM sodium chloride in the meal instead of 250 m-osM sodium chloride in the absence of glucose.

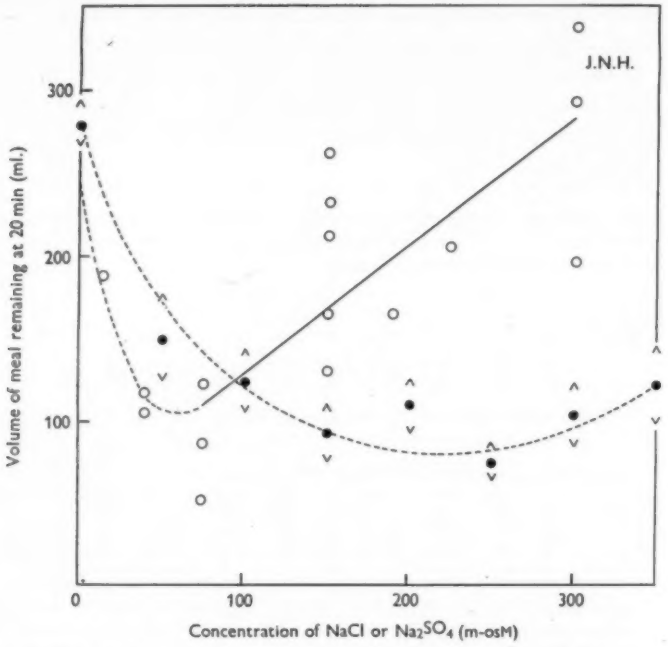

Fig. 2. A comparison of the influence of NaCl (●) and of Na_2SO_4 (○) on the volume of test meals remaining in the stomach after 20 min (J.N.H.). In all Figs. S.E. of means indicated by ⬍⬎.

Figure 4 shows some data from experiments designed to put the conclusions from Fig. 3 on a more secure basis. In this instance the recoveries were made after 20 min and the effect of 126 m-osM glucose and sodium chloride was compared with that of sodium chloride without glucose. The figure shows:

(1) That the concentration of sodium chloride giving a minimal recovery, that is, maximal rate of emptying, is less with 126 m-osM glucose than it is with sodium chloride alone;

(2) That the increase in the volume recovered per osmole increase in concentration of sodium chloride in the meal is less in the presence of glucose;

(3) That at high concentrations of sodium chloride the volume recovered

with the meals containing glucose was *less* than with the meals containing sodium chloride alone.

Altogether 306 test meals of this type were given to three subjects. In the other two the results were similar to those shown in Fig. 4, except that in one the lowering of the concentration of sodium chloride giving the maximal recovery in the presence of glucose was not shown.

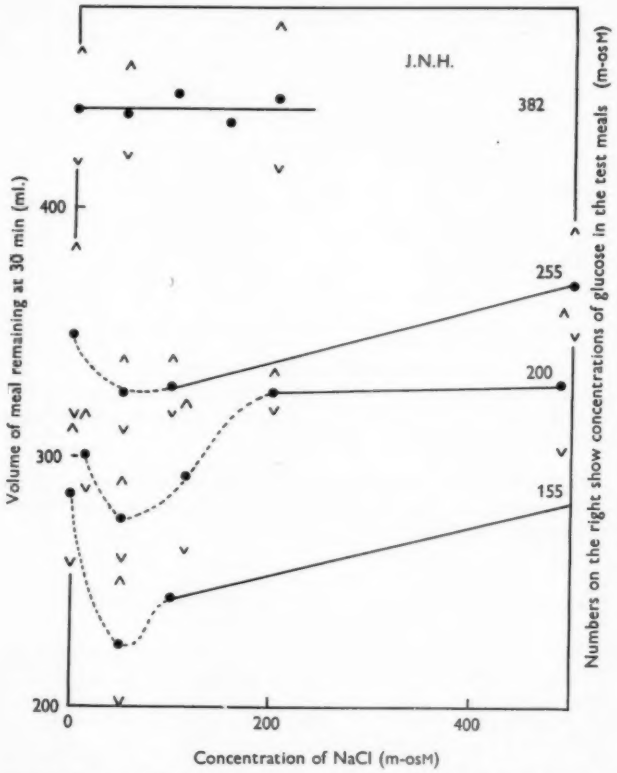

Fig. 3. The influence of different concentrations of glucose on the volume of saline solutions remaining in the stomach after 30 min (J.N.H.).

The relation between the concentration of sodium chloride and the volume of meal recovered was further studied with an experiment of the type illustrated in Fig. 3 in J.D.P. and J.N.H., with recoveries at 20 min after instillation. The slopes of the lines relating concentration of sodium chloride to volume recovered, over the range where the relationship seemed linear, are given in Table 2. The slopes became less as the concentration of

glucose was raised, as may be seen from Fig. 5, in which the slopes of the lines from Table 2 have been plotted against the relevant concentration of glucose. By extrapolation the concentration of glucose abolishing the effect of the concentration of sodium chloride on the volume of meal recovered was found to be 254 m-osm for J.N.H. and 270 m-osm for

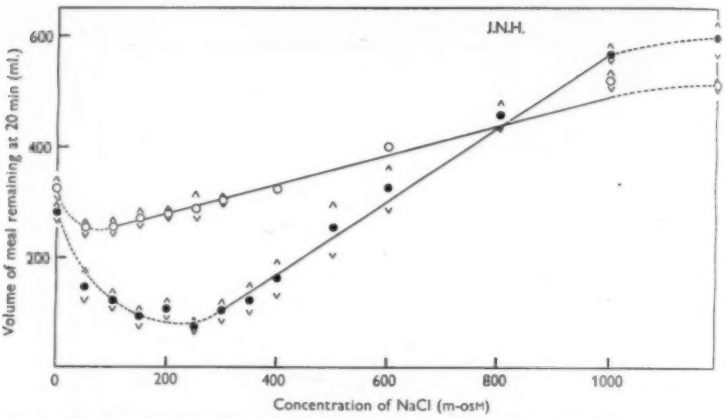

Fig. 4. The influence of the concentration of NaCl alone (●) and of NaCl in the presence of glucose 126 m-osm (○) on the volume of test meals remaining in the stomach after 20 min (J.N.H.).

Table 2. The influence of the concentration of glucose on the relationship between the volume of meal remaining in the stomach after twenty minutes and the concentration of sodium chloride in the test meal

Concentration of glucose (m-osm)	J.D.P. Range of concentrations of NaCl (m-osm)	n	a	b	s.e. of b	J.N.H. Range of concentrations of NaCl (m-osm)	n	a	b	s.e. of b
0	160–780	16	114	0·42	±0·07	350–1000	15	−105	0·68	±0·06
100	200–800	5	120	0·39	±0·09	150–800	7	124	0·385	±0·04
127	—	—	—	—	—	100–600	30	227	0·26	±0·055
155	190–810	8	265	0·16	±0·07	200–810	8	298	0·22	±0·06
200	200–800	12	370	0·17	±0·05	100–1000	10	377	0·18	±0·085
255	—	—	—	—	—	0–1000	10	505	0·036	±0·012

* y = volume of meal remaining at 20 min (ml.); x = concentration of sodium chloride (m-osm); n = number of observations; a corresponds to the volume of meal remaining with zero concentration of sodium chloride, that is the intercept of the regression line and the ordinate; b corresponds to the slopes of the lines similar to those shown in Fig. 3; $y = a + bx$.

J.D.P. When these values are adjusted for the osmotic coefficient of glucose they become 274 and 290, that is slightly less than isosmolar with plasma.

An interesting feature of this failure of the mechanism determining gastric emptying to respond to sodium chloride in the presence of glucose

can be appreciated by looking at Fig. 4. The volume of meal recovered with 1200 m-osM sodium chloride was *less* in the presence of 126 m-osM glucose than it was without the glucose, although in the absence of sodium chloride glucose *increased* the amount recovered. It seemed desirable to make a direct contrast between the influence of a constant concentration of glucose upon the response to a changing concentration of sodium chloride, and the influence of a constant concentration of sodium chloride

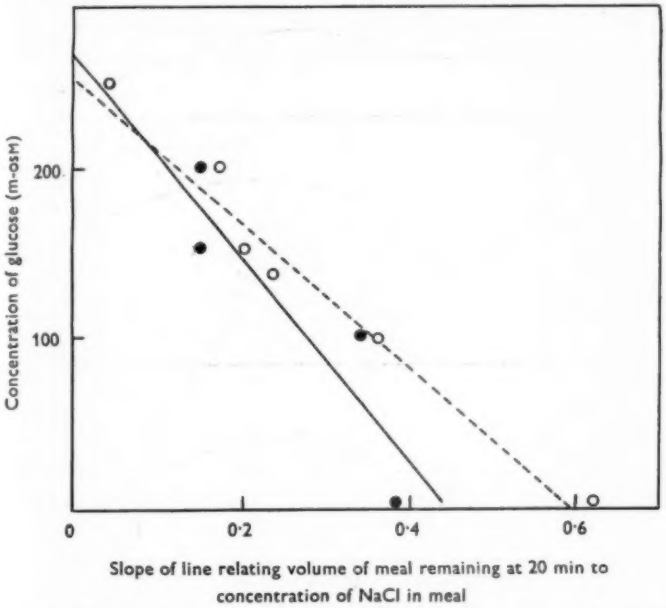

Fig. 5. The relation between the concentration of glucose in a test meal and the effect of NaCl on the volume of test meal remaining in the stomach after 20 min. ● J.D.P.; ○, J.N.H.

upon the response to a changing concentration of glucose, although the general form of the relationship at concentrations of sodium chloride up to 200 m-osM is apparent from Fig. 3. Eight test meals of 30 min duration, containing varying amounts of glucose and 500 m-osM sodium chloride, were given to J.N.H.: the slope of the line relating concentration of glucose to volume of meal recovered was 1·27, S.E. ± 0·27. There is thus no doubt that sodium chloride even at a m-osM concentration greater than that of the plasma did not abolish the slowing action of glucose on gastric emptying, whereas a solution of glucose isosmotic with plasma did abolish the slowing action of sodium chloride.

The influence of sodium chloride on gastric emptying in the presence of acid

Figure 6 shows the relationship based on 132 meals in three subjects between the concentration of sodium chloride in test meals and the volume

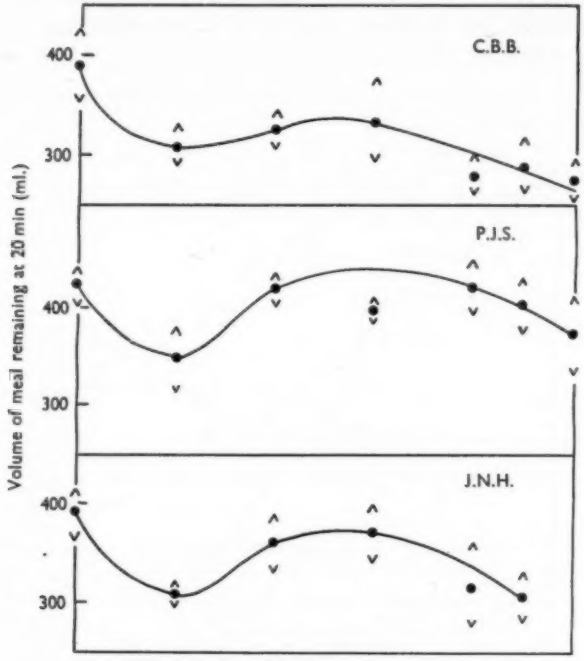

Fig. 6

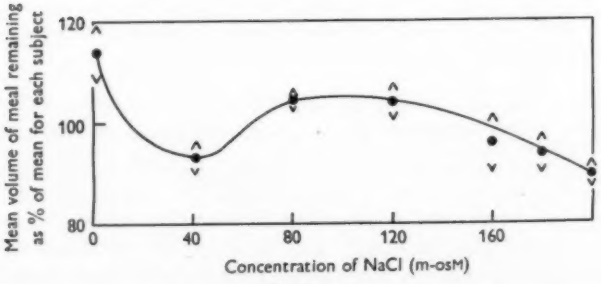

Fig. 6*a*

Fig. 6. The influence of sodium chloride in the presence of 40 m-osM HCl on the volume of test meals remaining in the stomach after 20 min (C.B.B., P.J.S. and J.N.H.); (6*a*) combined data of Fig. 6.

of meal recovered in the presence of 40 m-osM hydrochloric acid. These data were combined by expressing the volume of each recovery as a percentage of the mean volume of all recoveries for that subject. The means of these percentages for the three subjects are shown at the foot of Fig. 6. In the presence of 40 m-osM hydrochloric acid there was a lower recovery with the meal containing 40 m-osM sodium chloride than with immediately higher or lower concentrations of sodium chloride. With higher concentrations of sodium chloride the volume recovered fell as the concentration of sodium chloride was raised to 200 m-osM. It was not possible to explore the effects of higher concentrations of sodium chloride because they produced nausea.

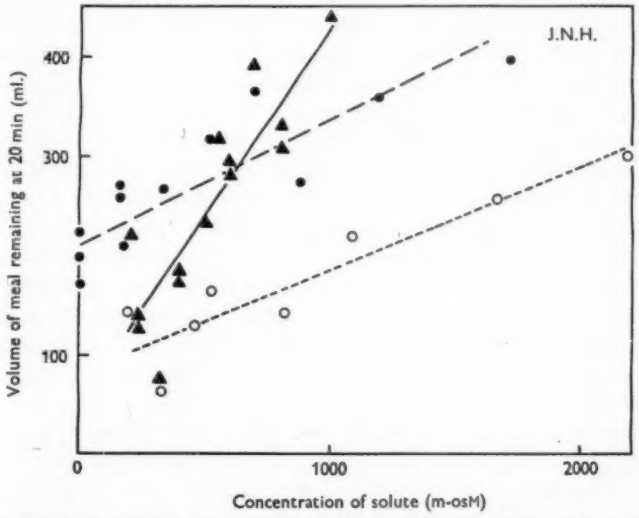

Fig. 7. The influence of ethanol (●), urea (▲) and glycerol (○) on the volume of test meals remaining in the stomach after 20 min (J.N.H.).

The influence of urea, ethanol and glycerol on the volume of test meal recovered after a fixed interval

Figure 7 shows data obtained in J.N.H. from test meals containing urea or glycerol. The response to test meals containing these solutes was similar to the response to meals containing sodium salts. There was a minimal recovery with 100–200 m-osM solutions. At higher concentrations these solutes gave progressively larger recoveries. Figure 7 also shows that with meals containing ethanol no minimal recovery was detected, the volume recovered increasing progressively as the concentration of ethanol in the meal was increased from zero.

DISCUSSION

The present experiments have confirmed the conclusions of earlier workers (Apperly, 1926; McSwiney & Spurrell, 1933; Shay, 1944) that there is a relationship between the concentration of a solute in a test meal and the rate of gastric emptying of the meal, and that this relationship varies with the solute concerned. The problem here is to describe the working of the receptor mechanism in this regulation of gastric emptying.

The rate of emptying of the stomach might be thought to depend upon the degree of distension of the duodenum or jejunum by the gastric effluent. If the original meal contained some solute which was very little absorbed, the volume of the contents of the sensitive segment might remain large as a result of the osmotic action of the solute, and gastric emptying would be slowed. Hypotheses of this kind appear to be made untenable by the equal action per osmole of calcium chloride and sulphates (Ingraham & Visscher, 1938), which are relatively little absorbed from the intestine, and glucose which is very readily absorbed. In addition, it has been found that when two meals are given in succession the second, passing into a full intestine, leaves the stomach more rapidly than the first (Hunt & Macdonald, 1954). Further, with meals of 750 ml. the 'half-life' of the emptying process is constant over the period during which the main part of the meal empties, but the fullness of the intestine must vary considerably during this time (Hunt & Macdonald, 1954). Taken together these facts suggest that some mechanism other than the fullness of the duodenum or small intestine controls gastric emptying under these conditions.

In the discussion which follows, the stimulus to the receptor mechanism has to be assumed to be adequately described by specifying the composition of the test meal. The experiments are designed to minimize the importance of any alimentary contributions to the environment of the receptor by washing out the stomach before the meal is given, by using a large volume of meal and by considering data mainly obtained from meals of short duration. At the time of the recovery the meal was usually diluted by about 10% as judged from the change in concentration of phenol red. There was no evidence that the highly concentrated solutions were more diluted in the stomach (Hunt, Macdonald & Spurrell, 1951), the greatest dilution occurring with meals which emptied rapidly, because of the smaller volume of the meal diluted by secretion and because secretion was less inhibited. At the time of the recovery the concentration in the gastric contents of acid derived from secretion was almost always less than 10 m-equiv/l., which is below the known threshold for effects on gastric emptying: and, finally, some of the receptors responding to solutes in the gastric contents probably lie just beyond the pylorus (Shay & Gershon-

Cohen, 1934), so that dilution by pancreatic secretion or bile might not influence the solution in contact with them. Considering all these points together, it may be concluded that although assessing the stimulus to the receptors from the composition of the instilled meal introduces an error it can be accepted for the moment. None of the test meals used in the experiments reported here produced nausea. Even had they done so it would not invalidate the results, since the work is concerned with the relationship between stimulus, receptors and response and not with the pathways mediating the response. It appears that the receptors of low threshold initiating the sensation of nausea lie in the walls of the duodenum (references cited by Hunt, 1956).

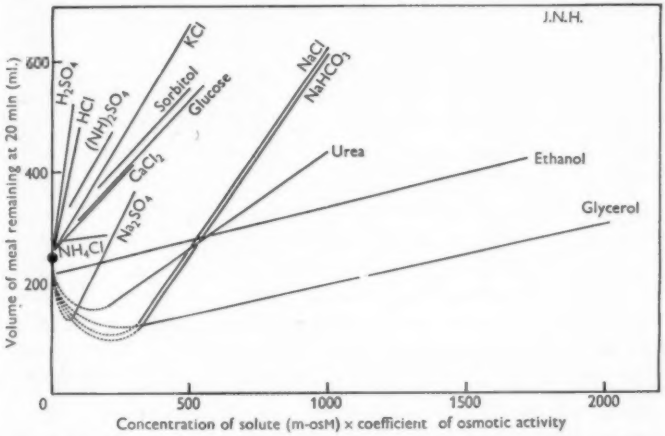

Fig. 8. The influence of the osmotic activity of various solutes on the volume of test-meal remaining in the stomach after 20 min (J.N.H.). Because Table 1 and Fig. 1, 2 and 4 include divergent data for eight meals of water obtained after the majority of the experiments in Fig. 8 were completed, the mean volume of meal of water recovered is not the same in Fig. 8 as in the other Figures. (On figure, for $(NH)_2SO_4$ read $(NH_4)_2SO_4$.)

It may be seen in Fig. 8 and Table 3 that the slopes of the lines relating the m-osM concentration of solute and the change in volume of meal recovered are approximately the same for ammonium sulphate, potassium chloride, sorbitol, glucose, calcium chloride and at high concentrations for sodium chloride, sodium sulphate and sodium bicarbonate. These compounds include combinations of monovalent and divalent anions and cations, and non-electrolytes, and it is difficult to envisage a separate receptor for each. On the other hand a single receptor responding to all more or less equally would presumably be sensitive to some common property: their osmotic activity is a candidate for consideration and there

is no obvious alternative. Hydrochloric and sulphuric acids are much more potent than the solutes mentioned above, so that they probably work via a different mechanism as they do in controlling gastric secretion in the dog (Sircus, 1958). It will be assumed that the osmotic regulation of emptying depends upon a single type of receptor, although at the moment there are no grounds, apart from the principle of Occam's razor, *entia non sunt multiplicanda praeter necessitatem*, for supposing that this is so.

TABLE 3. Relative activity of solutes on the osmoreceptor mechanism in one subject

Solute	a*	b	S.E. of b	n	Range of concentrations (m-osm)
H_2SO_4	242	2·60	±0·37	16	0–77
HCl	238	2·32	±0·11	43	0–100
HCl	180	3·07	±0·38	22	20–100
KCl	200	0·93	±0·25	21	40–500
KCl	257	0·75	±0·07	40	0–500
$(NH_4)_2SO_4$	279	0·64	±0·18	23	0–225
Glucose	260	0·57	±0·04	36	0–550
Sorbitol	281	0·50	±0·07	24	0–500
$CaCl_2$	260	0·44	±0·14	35	0–300
Ethanol	213	0·12	±0·02	12	0–1720
NH_4Cl	273	0·005	±0·18	26	0–200
Na_2SO_4	52	0·77	±0·26	13	75–300
$NaHCO_3$	−115	0·70	±0·11	12	320–1000
NaCl	−105	0·68	±0·06	15	350–1000
Urea	76	0·38	±0·10	14	200–1000
Glycerol	85	0·10	±0·02	8	330–2170

* y = volume (ml.) of 750 ml. meal remaining in the stomach 20 min after ingestion; x = concentration of solute (m-osm); n = number of observations; $y = a + bx$; a corresponds to the volume (ml.) of meal remaining at zero concentration of solute, that is the intercept of the regression line with the ordinate.

So far the effects of solutes on gastric emptying can be described and explained in seven sections which may be more readily understood when they are considered in conjunction with Table 3 and Fig. 8.

(1) Glucose, sorbitol, sulphates, salts of potassium, calcium and at high concentrations only, sodium, have about equal effects per osmole in slowing gastric emptying. They are postulated to reduce the volume of sensitive component of an osmoreceptor responding to the contents of the alimentary lumen beyond the pylorus. These solutes are thought to penetrate the selective membrane of the osmoreceptor slowly if at all. A signal from the osmoreceptor which slows gastric emptying is postulated to occur with test meals of pure water and to increase when the flux of water into the sensitive component is reduced by the osmotic action of solutes in the lumen.

(2) Ammonium, bicarbonate and chloride are postulated to penetrate the osmoreceptor freely by diffusion and to have no effect on gastric emptying except the inhibition by the water in which they are dissolved.

However, when any one of these ions is paired with any ion listed under 1, it is restrained from penetrating by the demands of electrical neutrality and has an action per osmole equal to any solute listed under 1. There is evidence to suggest that bicarbonate may penetrate more freely than chloride (Hunt, 1956).

(3) Ethanol and, at high concentrations, urea and glycerol, are postulated to penetrate the osmoreceptor more slowly than unrestrained chloride, bicarbonate or ammonium but more rapidly than the solutes listed under 1. As these non-electrolytes continue to have an action on the hypothetical osmoreceptor for up to 20 min, their steady-state distribution across the osmoreceptor membrane presumably sets up an osmotic gradient greater than that postulated for ammonium and bicarbonate, which reduces the flux of water into the receptor.

(4) At low concentrations sodium, urea and glycerol are postulated to be subject to either facilitated diffusion or active transport into the osmoreceptor, thus favouring the flux of water into the receptor and reducing the inhibitory action of the receptor which is postulated to occur with pure water.

(5) Solutions of glucose isosmotic with plasma are postulated to make the membrane of the osmoreceptor so permeable to sodium that it neither osmotically reduces the volume of the osmoreceptor at high concentrations nor increases it at low concentrations as a result of its supposed facilitated migration into the receptor.

(6) Strong acids and glucose are postulated to have different modes of action since hydrochloric acid and glucose have different effects on the response of the osmoreceptor to sodium chloride. Moreover, strong acids are about three times more effective per osmole than the next most powerful solutes in slowing gastric emptying. These views are in harmony with those of Sircus (1958) on the control of gastric secretion.

(7) The increased rate of emptying with low concentrations of sodium sulphate may possibly be the result of the presence of bicarbonate or chloride ion secreted from the pancreas. At higher concentrations of sulphate the effect of the chloride may be masked.

In devising further studies of the supposed osmoreceptor the red cell, which has been studied as an osmometer by Ponder (1940), may provide a useful model. Many of the properties postulated for the osmoreceptor have been demonstrated for red cells: for example, facilitated migration of glycerol (LeFevre, 1948) and sodium (Davson & Reiner, 1942); increased permeability to sodium on exposure to hypertonic solutions (Davson & Reiner, 1942); penetration by ammonium (Jacobs & Parpart, 1938); slower penetration by sulphate than by chloride (Davson, 1959, from data of Luckner, 1948) and restraint of penetration of chloride and bicarbonate

by potassium, sodium or calcium (Ponder, 1953) are accepted. At high concentrations the red-cell membrane behaves as though it were impermeable to glucose (Widdas, 1954). It is interesting that the very indirect approach via studies of gastric emptying can lead to postulates so similar to the known facts about red cells.

SUMMARY

1. The influence on gastric emptying of several solutes in test meals was studied by plotting the volume of test meal remaining after a fixed interval against the osmolar concentration of the solute in the original meal.

2. On an osmolar basis hydrochloric acid and sulphuric acid were about three times as potent as potassium chloride in slowing gastric emptying.

3. Ammonium sulphate and calcium chloride were two to four times more potent than ammonium chloride in slowing gastric emptying.

4. At low concentrations the addition of sodium chloride and sodium sulphate to test meals increased the rate of gastric emptying. The osmolar concentration in the meal giving a maximal rate of emptying was less for sodium sulphate than for sodium chloride. At high concentrations sodium sulphate and sodium chloride were about equally potent in slowing gastric emptying.

5. Increasing the concentration of glucose in test meals containing sodium chloride progressively reduced the action of sodium chloride on gastric emptying. At concentrations of glucose nearly isosmotic with plasma sodium chloride lost all its influence on gastric emptying. In contradistinction, solutions of sodium chloride isosmotic with plasma did not abolish the inhibitory action of glucose on gastric emptying.

6. In the presence of 40 m-osM hydrochloric acid low concentrations of sodium chloride increased the rate of gastric emptying as they did in the presence of equipotent concentrations of dextrose. A complex relationship between concentration of sodium chloride and the rate of gastric emptying in the presence of acid was defined.

7. Urea and glycerol in test meals hastened gastric emptying at low concentrations but slowed it at high concentrations. Ethanol slowed gastric emptying as the concentration was increased.

8. The findings are explained on the assumption that there is an osmoreceptor mechanism in the intestine which inhibits gastric emptying. The majority of solutes are assumed not to penetrate the receptor, but sodium, urea and glycerol are thought to be subject to facilitated transport across the osmotic membrane at low concentrations and urea and glycerol to slow diffusion at high concentrations. Solutions of glucose isosmotic with plasma are thought to make the supposed osmoreceptor permeable

to sodium. The action of acids is thought to depend upon a separate mechanism.

We are greatly indebted to the students who acted as subjects in this work, without whose unfailing co-operation it would have been impossible. It is a pleasure to acknowledge help received in discussion with Drs H. Davson and W. F. Widdas. We are very grateful to Professor W. R. Spurrell for much improving the manuscript.

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THE SITE OF RECEPTORS SLOWING GASTRIC EMPTYING IN RESPONSE TO STARCH IN TEST MEALS

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There is a body of evidence consistent with the view that the osmotic pressure of the gastric content is important in determining its rate of emptying into the duodenum (Carnot & Chassevant, 1905; Apperly, 1926; McSwiney & Spurrell, 1933; Gershon-Cohen, Shay & Fels, 1938; Hunt, Macdonald & Spurrell, 1951; Hunt, 1956; Hunt & Pathak, 1960). The receptor mechanism involved in this regulation of gastric emptying might respond to the osmotic pressure of the gastric content as it lay in the stomach, or it could work equally well if it detected the osmotic pressure of what was recently gastric content but actually occupied the lumen of the duodenum or small intestine. The duodenal and intestinal receptors can operate in the osmotic regulation of gastric emptying because it is slowed by instilling solutions hyperosmotic to plasma into the duodenum (Shay, Gershon-Cohen, Fels & Siplet, 1942).

If the osmotic pressure of the gastric contents acting on gastric receptors were to slow gastric emptying, it would be expected that test meals containing starch, with very low osmotic pressure, would leave the stomach quickly, whereas test meals containing an equal amount of carbohydrate as glucose would stimulate the osmoreceptors and the stomach would empty slowly. Alternatively, if the osmoreceptors were in the duodenum distal to the site of hydrolysis of starch, meals containing either starch or glucose would be expected to empty from the stomach at equal rates. This paper contains an account of some experiments designed to decide between these alternatives.

METHODS

Test meals were given to healthy volunteer medical students of both sexes who came to the laboratory in the morning fasting. The stomach having been washed out with 250 ml. of tap water, test meals at 37° C were instilled down a tube into the stomach. After varying periods of time the gastric contents were recovered and the stomach was washed out with 250 ml. of tap water. Each test meal contained in addition to the carbohydrate as the experimental variable about 60 mg phenol red/l.: this is known not to be absorbed from the stomach and to have no significant effect on gastric emptying (Hunt, 1956). This phenol red served as a marker so that the volume of the original test meal recovered from the stomach could be calculated from the total amount of phenol red recovered by dividing it

by the concentration of phenol red in the original test meal. The amounts of acid and chloride secreted in response to the meals were also calculated by methods which have been described (Hunt, 1951; Hunt & Spurrell, 1951).

Each subject was given one test meal for practice before the main series was begun. All the test meals were of 750 ml. except those given to subject LO which were 500 ml., as she weighed only 46 kg. The meals were given on successive days, keeping the period of experimentation as short as possible to minimize the effect of any tendency to changes in gastric emptying with time. Meals containing glucose were prepared from dextrose monohydrate B.P. Analar soluble starch was weighed out after determining the water content as loss of weight after 1 hr at 105° C. The meals were made up immediately before the experiments by mixing the starch with cold water and pouring the suspension into boiling water, bringing it to the boil and then cooling it by adding ice cubes. It was then made up to volume with distilled water and the stock solution of phenol red. This procedure was necessary because it was not possible to instil the starch solution down a tube once it had been allowed to cool slowly and form a gel. The calorific value of starch was taken as 4·2/g and that of glucose as 3·8/g.

RESULTS

Comparison of the gastric responses to test meals containing isocaloric amounts of starch or glucose

Three women and six men were given a total of 78 test meals. The volumes of the original test meals recovered at intervals after their ingestion are shown in Fig. 1 for four subjects, the data being chosen to show the full diversity of responses obtained. The ordinate, showing the volume of original meal remaining in the stomach, is plotted on a logarithmic scale. This allows straight lines to be fitted to the data excluding those for the volume of meal ingested, since the rate of emptying of the meal excluding the first few minutes is a constant fraction of the volume of the meal remaining in the stomach (Marbaix, 1898; de Salamanca, 1943; Hunt & Spurrell, 1951). The concentration of carbohydrate in the test meals was 36 g glucose/l., isocaloric with 33 g dry starch/l. for subjects, LO, DI, PN, GE, JEN and 45 g glucose and 41 g dry starch/l. for CE, HE and JON. From the lines fitted as shown in Fig. 1 the time required for the volume of the meal remaining in the stomach to fall by half, the 'half-life', can be calculated. The half-lives of the test meals of pure water and those containing either starch or glucose are given in Table 1. It may be seen that in six subjects out of nine the half-life of the test meal of water was only about 50% of the value for test meals containing carbohydrate. Nevertheless, the mean difference between the half-lives for the meals containing starch and those containing dextrose was only 3·6 min, S.E. $\pm$ 2·6 ($P = 0{\cdot}2$), so that by this criterion starch and glucose were equally effective in slowing gastric emptying.

Table 1 also shows that the volume of the test meal containing starch remaining in the stomach 20 min after its instillation was less ($P = 0{\cdot}01$) than the volume of the test meal containing glucose remaining at this time.

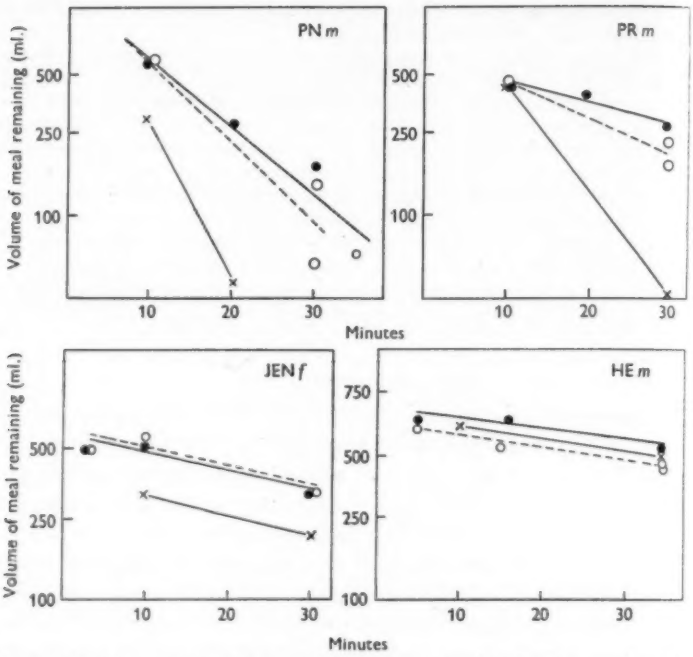

Fig. 1. Gastric emptying of test meals of water (×), and of solutions of starch (○) or glucose (●). Ordinate, log. scale.

TABLE 1. The influence of isocaloric concentrations of glucose or starch in test meals on gastric emptying

	Half-life (min)				Volume of meal remaining at 20 min (ml.)		
Subject	Glucose, *A*	Starch, *B*	Water	*A*−*B*	Glucose, *C*	Starch, *D*	*C*−*D*
LO *f*	20·8	20·4	10·6	+ 0·4	258	232	26
DI *m*	34·2	24·0	13·3	+10·2	412	398	14
PN *m*	8·9	7·6	3·5	+1·3	271	225	46
GE *f*	28·3	37·5	7·5	−9·2	476	407	69
PR *m*	30·0	17·1	5·7	+12·9	347	288	59
JEN *f*	34·1	32·2	31·5	+1·9	411	431	−20
CE *m*	27·0	31·5	50·8	−4·5	445	404	41
HE *m*	65·2	58·7	53·5	+6·5	658	547	111
JON *m*	62·5	50·0	13·2	+12·5	555	417	138
			Mean	3·6		Mean	54
				S.E. ±2·6			S.E. ±16

f, female; *m*, male. *C* and *D* were calculated from regression equations.

It seems likely that there was an initial phase of gastric emptying, during which test meals containing starch left the stomach more quickly than test meals containing glucose, but subsequently the rates of emptying for the two types of meal were similar. The data of Fig. 1 were chosen to show the diversity of the responses and there happens to be only one clear example of the more rapid initial emptying of the meal of starch.

Under the conditions of these experiments, in which the meal was instilled into the stomach and the subjects did not swallow their saliva, there was very little hydrolysis of starch in the stomach. The depression of freezing point of the original meals containing starch was 0·01° C. After 30 min gastric digestion the depressions of freezing point of three gastric contents with meals containing starch was 0·05, 0·10 and 0·10° C but the ions of gastric secretion accounted for 0·04, 0·08 and 0·09° C respectively. Thus, as judged from the depression of freezing point, the concentration of glucose in the gastric contents 30 min after taking the test meals of starch solution was very small. A meal containing such a concentration of glucose without starch would empty at the same rate as pure water.

It is known that the greater the acidity of the gastric contents the slower is the rate of gastric emptying (Pathak, 1959). The question therefore arises as to whether the approximate equality of rates of emptying of the meals containing starch or glucose results partly from a greater acidity of the gastric contents with the test meal containing starch. There was in fact a slightly greater secretion of acid with the test meal containing starch than with the test meal containing glucose, 1·18 m-equiv H^+/30 min, S.E. $\pm$ 0·40, $P = 0·02$. However, in many instances the acidity of the gastric contents did not reach 5 m-equiv/l. whilst the threshold concentration for the effect of acid on gastric emptying is not less than 10 m-equiv/l. (Hunt, 1956; Pathak, 1959).

DISCUSSION

The hypothesis that gastric emptying is dependent upon the osmotic pressure of the gastric contents has emerged from the cited experiments with test meals containing a wide variety of electrolytes and non-electrolytes. However, in the experiments described above it was found that test meals containing starch, with a very low osmotic pressure, and those containing an equal amount of carbohydrate as glucose, with an osmotic pressure many times greater, had equal half-lives. As in most instances test meals of water left the stomach more quickly than the meals with carbohydrate it is clear that the starch and the glucose were both slowing gastric emptying. The equality of the rates of emptying cannot be ascribed to the greater viscosity of the meals containing starch, since

differences of viscosity of the order of that existing between the meals containing glucose and those containing starch have been found not to influence gastric emptying significantly (Hunt, 1954). The simplest way of harmonizing these observations with the hypothesis of osmotic control of gastric emptying is to suppose that the starch in the test meal was hydrolysed before it was presented to the receptors which responded to the osmotic pressure. Since in the present experiments, in contrast to those of Beazell (1941) under more normal conditions, there was very little hydrolysis of starch in the stomach, the results make it probable that the receptors responding to starch lie at or beyond the pylorus. This conclusion is in agreement with that of Shay *et al.* (1942), who found that the slowing of emptying was produced by instilling solutions hyperosmotic to plasma into the duodenum. Although the slowing of emptying due to *starch* seems to depend upon receptors at or beyond the pylorus, there is nothing in the present experiments to exclude the possibility of there being receptors in the gastric mucosa which respond to *glucose*.

The finding of a response by the supposed osmoreceptors to solutions of starch allows the receptor to function when carbohydrate is eaten as starch, rather than as sugar. That the volume of the meal remaining in the stomach at 20 min was greater with glucose than with starch is explained by supposing that the meal containing starch initially left the stomach more quickly than the meal containing glucose. This is consistent with the idea that the slowing of gastric emptying depended upon the hydrolysis of the starch in the meal and that until this had occurred the emptying of the meal with starch was more rapid than that of the meal containing glucose.

It is interesting to consider what part these receptors may play in the regulation of other activities besides gastric emptying. It has been found that in rats when solutions of high osmotic pressure are given intragastrically the intake of food is subsequently reduced (Schwartzbaum & Ward, 1958). Furthermore, substances which on an osmolar basis slow gastric emptying equally in man, glucose and sorbitol (Hunt, 1956), are equally effective when instilled into the stomach in suppressing the appetitive behaviour of rats. On the other hand under the same conditions glucose is more effective than glycerol, both in slowing gastric emptying (Hunt & Pathak, 1960) and in suppressing appetitive behaviour (Smith & Duffy, 1955). It is not clear whether these effects are mediated directly from the osmoreceptors or whether they depend upon the increased volume of the gastric contents which results from the slowing of gastric emptying.

In spite of the equal half-lives of meals containing either starch or glucose, the amount of acid secreted was less after the meal containing

glucose. Possibly this may depend upon a slightly earlier onset of inhibition via the duodenum with the meal containing glucose.

SUMMARY

1. Test meals containing isocaloric amounts of carbohydrate as glucose or as starch were given to six men and three women.

2. Test meals of water emptied more rapidly than the meals containing carbohydrate, with the implication that the starch and glucose both excited receptors which slowed gastric emptying.

3. The half-lives, excluding the first few minutes, of the meals containing either starch or glucose were similar. Twenty minutes after instillation the intragastric volume of the meal containing starch was less than that for the meal containing glucose. Taken together these two findings suggest that initially the meal containing starch left the stomach more rapidly than the meal containing glucose but subsequently the rates were equal.

4. There was a significantly larger secretion of acid with the test meal containing starch compared with the one containing glucose. The concentrations of acid in the gastric contents were too low to influence gastric emptying.

5. These findings were taken to be consistent with the hypothesis that the slowing of gastric emptying with test meals containing starch was the result of stimulation of osmoreceptors beyond the site of hydrolysis of the starch. As the starch in the main part of the gastric contents was unhydrolysed the receptors presumably lay at or beyond the pylorus.

I am very grateful for the co-operation of the students who took part in these experiments.

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INCREASE IN NORADRENALINE CONTENT OF TISSUES AFTER INFUSION OF NORADRENALINE, DOPAMINE AND L-DOPA

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(*Received* 21 *April* 1960)

The responses to tyramine and to sympathetic stimulation are reduced or abolished when the noradrenaline content of the tissues has been depleted by reserpine treatment (Carlsson, Rosengren, Bertler & Nilsson, 1957). They are restored after an infusion of noradrenaline (Burn & Rand, 1958, 1960*a*), or infusion of precursors of noradrenaline, such as dopamine (*β*-3: 4-dihydroxyphenylethylamine) and L-DOPA (Burn & Rand, 1960*b*). These observations led to the suggestions that it is the store of noradrenaline in a tissue which is necessary for the response to tyramine and that the size of the store determines the magnitude of the response to sympathetic stimulation. Our purpose in the present paper has been to determine the effect of infusions of noradrenaline, dopamine and L-DOPA in increasing the store of noradrenaline in organs of normal and reserpine-treated cats.

METHODS

Spinal cats (2·5–3·5 kg) were prepared by Dale's method as described by Burn (1952). Evisceration was performed by dividing the following structures between ligatures: rectum, inferior and superior mesenteric arteries, coeliac axis, oesophagus, portal vein and bile duct. Blood pressure was recorded from one carotid artery and a 1 mm polythene tube was placed in the other carotid artery to withdraw blood samples. The right kidney was dissected free from fat and removed after ligating the vessels at the hilum. In female cats one horn of the uterus was tied close to the fundus and the horn was removed after ligating the ovarian artery and vein. A sample of 5–6 ml. of blood was withdrawn into a polythene centrifuge tube containing 100 u. heparin. Noradrenaline (1 mg), dopamine (25 mg) or L-DOPA (25 mg) was infused into a small vein in the foreleg from a continuous slow-injection syringe (C. F. Palmer Ltd.). Each drug was given in 14·3 ml. of 0·9 % NaCl adjusted to pH 4–5 and was infused during 40 min. The infusion apparatus was then disconnected and after a further 20 min had elapsed the left kidney and the other uterine horn were removed and a second blood sample was taken. At this time the blood pressure had fallen from the level attained at the end of the infusion and had remained at a low steady value for 10–15 min.

The blood samples were centrifuged for 10 min at 5000 rev/min immediately after collec-

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tion and the decanted plasma stored at −10° C until required for assay. The capsule was removed from the kidney and the uterus freed from connective tissue. These organs were dried between filter papers, weighed, and stored in the deep freeze. The total time which elapsed between removal of the organs and placing them in the deep freeze was 10 min. To determine the noradrenaline content of tissues they were extracted in 5 ml. of saline/g by the method described by Burn & Rand (1959) and assayed on the blood pressure of the pithed rat, recorded by inserting into the carotid artery a polythene tube connected to a strain-gauge pressure transducer (Statham Laboratories type P 23 Db). The output from the transducer bridge circuit was fed into a DC amplifier connected to an ink-writing recorder.

Cats were treated with reserpine by intraperitoneal injection of 3–4 mg of reserpine dissolved in 20% ascorbic acid on each of two days and were used for experiment on the third day.

Observations were made on the response of the cat's uterus to hypogastric nerve stimulation as described by Burn & Rand (1960*b*).

RESULTS

Assay of noradrenaline

Previous experience with the assay of organ extracts for noradrenaline on the blood pressure of the pithed rat has been virtually without complications arising from the presence of interfering pressor substances other

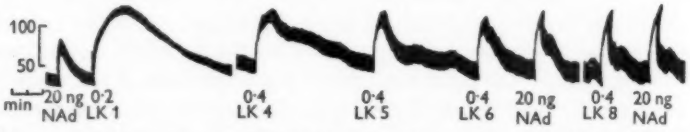

Fig. 1. Rat blood pressure recorded with a transducer manometer. LK 1–8, effect of successive injections of a left kidney extract, showing tachyphylaxis. LK 1, first injection (0·2 ml., i.e. ≡ 40 mg) produced a pressor response greatly exceeding in amplitude and duration that due to 20 ng noradrenaline (NAd). The 8th injection (LK 8, 0·4 ml.) produced a response virtually indistinguishable in form from that due to 20 ng noradrenaline. Pressure calibrations in mm Hg.

than noradrenaline. This was not the case when we injected extracts of kidney. The first injection of kidney extract into a pithed rat resulted in a large and prolonged rise of blood pressure. With subsequent injections of the kidney extract this slow pressor response became less and less marked and when the 4th–8th injections were given the blood pressure response was indistinguishable in form from that to an 'equi-pressor' dose of noradrenaline. Thus in Fig. 1 the first injection of 0·2 ml. of kidney extract (equivalent to 40 mg of kidney) produced a larger and longer-lasting response than 20 ng noradrenaline. However, the response to the 8th injection of the same extract (0·4 ml., equivalent to 80 mg of kidney) showed a complete tachyphylaxis to the slow pressor component and was now virtually indistinguishable from the response to 20 ng noradrenaline. After desensitizing the rat to the slow pressor component of the kidney extract we have regularly observed in these experiments a larger response

to noradrenaline. Our mean value for the noradrenaline content of normal cat's kidney was 196 ng/g (16 experiments, range 125–325 ng/g), which is very close to the figures published by von Euler (1956) who found a mean of 210 ng/g (4 experiments, range 110–320 ng/g). No difficulties were experienced in the assay of the noradrenaline content of plasma or of uterine extracts.

Increase in noradrenaline content after infusing noradrenaline

All the experiments with infusions of noradrenaline were carried out on eviscerated cats. In two preliminary experiments it was found that the noradrenaline content of a kidney removed immediately after preparing the cat was the same as the content in the second kidney removed 75 min later.

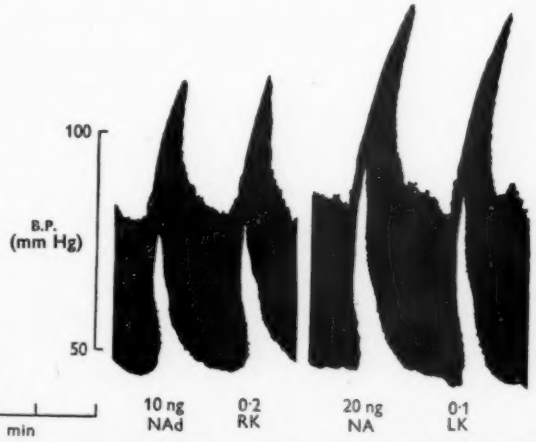

Fig. 2. Rat blood pressure. Uptake of noradrenaline by kidney from infusion of 1 mg noradrenaline. RK, response to 0·2 ml. of an extract of the right kidney (removed from the cat before infusion), equal to 10 ng noradrenaline (NAd). LK, 0·1 ml. of a similarly prepared extract of the left kidney, removed from the cat 20 min after the infusion.

The estimation of the pressor activity of extracts of kidneys taken from the same cat before and after the infusion of noradrenaline is illustrated in Fig. 2. In this experiment 0·1 ml. of the kidney extract made after the infusion had twice the potency of 0·2 ml. of control kidney extract; this represents a fourfold increase in content of noradrenaline.

The noradrenaline contents of the kidneys, uterine horns and blood plasma before and after the infusion of 1 mg of noradrenaline into both reserpinized and untreated cats are given in Table 1. In one untreated cat there was no increase in the noradrenaline content of the uterus and in

another neither the kidney nor the uterus took up noradrenaline from the infusion; it is interesting to note that the initial noradrenaline contents of these organs were the highest that were observed in this series of experiments. Uptake of noradrenaline was observed in every kidney and uterus of reserpine-treated cats. However, the mean figures for the content of noradrenaline in kidney and uterus after the infusion were lower in reserpine-treated than in normal cats. This observation suggests that reserpine treatment may inactivate part of the binding mechanism for noradrenaline.

TABLE 1. Noradrenaline content of kidney, uterus (ng/g) and plasma (ng/ml.) before and after infusing 1 mg noradrenaline

Kidney			Uterus			Plasma		
Before	After	Increase	Before	After	Increase	Before	After	Increase
A. Untreated, eviscerated cats								
188	250	62	—	—	—	—	—	—
250	500	250	1250	1250	0	—	—	—
162	325	163	500	1000	500	—	—	—
125	312	187	325	450	125	25	38	13
250	1000	750	625	1000	375	25	68	43
275	275	0	750	750	0	30	45	15
Means								
208	444	235	690	890	200	27	50	23
B. Reserpine-treated, eviscerated cats								
63	175	112	50	188	138	33	33	0
75	200.	125	38	188	150	25	38	13
250	750	500	188	253	65	75	75	0
150	250	100	100	188	88	38	100	62
Means								
135	345	210	94	204	110	43	57	14

The plasma pressor activity 20 min after the end of the infusion was raised in both normal and reserpine-treated cats. However, this increase in plasma noradrenaline could only contribute a very small part of the increases seen in the kidney and uterus.

The noradrenaline content of the uterus and the kidney was clearly increased after infusing 1 mg noradrenaline into cats. Raab & Gigee (1955, 1958) found that after the intraperitoneal injection of noradrenaline 10 mg/kg into dogs there was a mean increase of noradrenaline of 1·4 μg/g heart muscle and that the total catecholamine content of blood vessels (measured colorimetrically) was increased. However, von Euler (1956) stated that he could not significantly alter the catecholamine content of heart, spleen, liver, kidney or skeletal muscle of the cat by an intravenous infusion of 0·5 mg noradrenaline or by 1–2 mg/kg intraperitoneally. He considered that the results of Raab & Gigee were due to the use of massive doses (10 mg/kg intraperitoneally). The doses of noradrenaline used by us (7·1–10 μg/kg/min) were similar to von Euler's (2·5–7·8 μg/kg/min).

The estimation of plasma pressor activity in terms of noradrenaline in the pithed rat gave values which are higher than those generally accepted. However, it can be noted that Brown & Gillespie (1957) and Brown, Davies & Gillespie (1958), who used a similar procedure

for estimating plasma noradrenaline, published figures which are in accord with our results. Plasma from reserpine-treated cats had a higher pressor activity (in terms of noradrenaline). Muscholl & Vogt (1957) found an increase in the adrenaline level of plasma of rabbits after treating them with reserpine.

Infusion of dopamine

The results of infusing 25 mg dopamine into reserpinized and untreated eviscerated cats are given in Table 2. The extracts of the organs taken after the infusion gave an increased pressor activity on the pithed rat in only 1 out of 5 experiments with the normal kidney and in 3 out of 5 experiments with the normal uterus after the infusion of dopamine. Reserpine-

TABLE 2. Noradrenaline equivalent of kidney, uterus (ng/g) and plasma (ng/ml.) before and after infusing 25 mg dopamine

Kidney			Uterus			Plasma		
Before	After	Increase	Before	After	Increase	Before	After	Increase
			A. Normal, eviscerated cats					
200	200	0	250	250	0	25	40	15
188	188	0	188	250	62	38	38	0
125	125	0	375	375	0	25	59	24
300	625	325	1200	1300	100	25	90	65
188	188	0	200	262	62	25	75	50
Means								
200	265	65	443	487	44	28	60	31
			B. Reserpine-treated, eviscerated cats					
100	150	50	0	50	50	38	38	0
175	438	263	—	—	—	50	38	−12
150	250	100	100	188	88	38	100	62
112	112	0	188	313	125	18	58	40
75	125	50	38	63	25	13	38	25
Means								
122	215	93	82	154	72	31	54	23

treated cats more regularly showed an increase in noradrenaline content; uptake was observed in the kidney in 4 out of 5 experiments, and in the uterus in 4 of 4 experiments.

With our methods it was not possible to say directly whether the pressor activity on the pithed rat of the extracts made from the kidney and uterus after dopamine infusion was due to unchanged dopamine or to noradrenaline, which has served as the assay standard. In our hands dopamine has 1/200th of the pressor action of noradrenaline in the pithed rat (cf. 1/150, Vogt, 1959; 1/80, Muscholl, 1959). We observed a mean increase of 93 ng/g in the noradrenaline equivalent of reserpine-treated kidneys following dopamine infusion. If all the increased pressor activity were due to unchanged dopamine this would represent an uptake of 18·6 μg dopamine/g tissue. The most likely possibility is that part of the increase

in the pressor activity of the tissue extract was attributable to noradrenaline and part to unchanged dopamine.

Restoration of hypogastric nerve effect in reserpine-treated cats

Burn & Rand (1960*b*) have shown that an infusion of noradrenaline enhances the inhibitory response of the uterus to stimulation of the

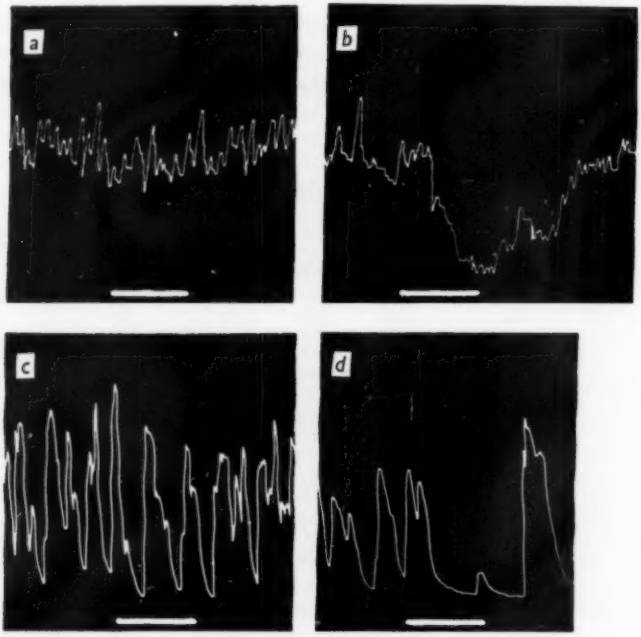

Fig. 3. *In situ* record of uterine movements in a reserpine-treated cat. Upper and lower records are from two separate experiments. The horizontal lines indicate stimulation for 2 min of the hypogastric nerve with square waves of 1 msec duration and 25 V amplitude at 20/sec. The inhibitory response to sympathetic nerve stimulation was restored by an infusion of 1 mg noradrenaline between (*a*) and (*b*), and of 25 mg dopamine between (*c*) and (*d*).

hypogastric nerve. Similarly an infusion of dopamine into a reserpine-treated cat restored the uterine response to hypogastric nerve stimulation (Fig. 3*d*). It is difficult to assess the relative efficacy of 1 mg noradrenaline and 25 mg dopamine in restoring the uterine response in reserpine-treated cats, since this response depends on the pattern of uterine activity. The upper portion of Fig. 3 shows a uterus with a rapid rhythm and a high tone. At first, stimulation had no effect, but 30 min after 1 mg noradrenaline

had been infused hypogastric nerve stimulation produced a decrease in tone (*b*). The lower records show another experiment in which the uterus had a slow rhythm of large amplitude which was not affected by nerve stimulation at first, but after infusion of 25 mg dopamine hypogastric stimulation resulted in an inhibition of rhythm (*d*).

Infusion of L-*DOPA*

Four experiments were carried out in which 25 mg of L-DOPA was infused during 40 min into eviscerated cats, two of which were untreated and two reserpine-treated. In no experiment was there any increase in the

TABLE 3. Noradrenaline content of kidney, uterus (ng/g) and plasma (ng/ml.) before and after infusing 25 mg L-DOPA

Kidney			Uterus			Plasma		
Before	After	Increase	Before	After	Increase	Before	After	Increase
			A. Normal, uneviscerated cats					
325	325	0	500	500	0	25	75	50
188	376	188	415	415	0	5	18	13
125	125	0	250	375	125	10	10	0
Means		63			42			21
			B. Reserpine-treated, uneviscerated cats					
125	188	63	63	125	62	30	40	10
175	225	50	50	92	42	13	13	0
88	63	−25	31	31	0	—	—	
Means		29			35			5

extractable pressor activity of kidney or uterus although the plasma activity was slightly increased. Six further experiments with L-DOPA were carried out on cats which were not eviscerated (3 untreated, 3 reserpine-treated). Uptake of noradrenaline was demonstrable in 4 cats (Table 3).

The spleens were removed from intact cats at the end of the experiment after infusing L-DOPA, and assayed for noradrenaline. The noradrenaline content of spleens after infusing L-DOPA, and noradrenaline content of spleens taken from cats at evisceration are given below:

Normal cat	1·0 μg/g
Normal cats after infusing 25 mg L-DOPA	0·875, 1·0 μg/g
Reserpine-treated cat	0·063 μg/g
Reserpine-treated cats after infusing 25 mg L-DOPA	0·375, 0·027, 0·213 μg/g

The values for the noradrenaline content of spleens from normal and reserpine-treated cats are in accord with those obtained elsewhere using identical methods (Burn & Rand, 1959). The infusion of L-DOPA did not increase the noradrenaline content of spleen from normal cats, but in 2 out of the 3 reserpine-treated cats the noradrenaline content was increased, by the infusion of L-DOPA, to 178 and 312 % of the mean value for reserpine-treated spleens. Von Euler & Uddén (1951) reported that the infusion of 5·2 mg of L-DOPA to 32 mg DL-DOPA

approximately doubled the noradrenaline content of the spleen, heart and liver. In their experiments the organs were removed immediately at the end of the infusion, whereas in ours 20 min elapsed.

It was apparent from these results that the presence of the abdominal viscera was necessary if the infusion of L-DOPA was to lead to an increase in the noradrenaline content of the tissues. We have examined this in the following way. Burn & Rand (1960*b*) showed that in reserpine-treated rats, in which the response to tyramine was small, an infusion of L-DOPA led to an increase in the response to tyramine. If the restored response to tyramine were due to a restoration of the noradrenaline content of the tissue, there should be a difference in the effect of an infusion of L-DOPA on the subsequent response to tyramine in non-eviscerated and eviscerated reserpine-treated cats. Two experiments were carried out to investigate this point. In both uneviscerated and eviscerated cats the infusion of noradrenaline led to an enhancement of the response to tyramine. In an uneviscerated cat there was a slight, but quite definite, increase in the response to tyramine after the infusion of L-DOPA, and this enhanced response was still seen for the third injection of tyramine after the infusion. On the other hand, in an eviscerated cat the infusion of L-DOPA brought about only a barely perceptible increase in the response to tyramine and the second injection of tyramine was again without action.

The infusion of L-DOPA at the rate of 25 mg in 40 min increased the blood pressure of the reserpine-treated uneviscerated cats. In four experiments the maximum blood pressure rises were 84, 80, 24 and 20 mm Hg. In reserpine-treated, eviscerated cats and in untreated cats L-DOPA had no pressor action. Clark (1959) states that complete evisceration usually decreases the response to L-DOPA, but not always.

DISCUSSION

The mean uptake of noradrenaline from an infusion of 1 mg was approximately 0·2 μg/g of tissue. In our experiments the cats were made spinal and eviscerated, and one kidney was removed before the infusion. The mean weight of the remaining kidney and uterus was 10 g, which would account for 2 μg of the noradrenaline infused. If one allows a weight of 20 g for the remaining sympathetically innervated structures (heart, lungs, blood vessels, etc.), and the same uptake per gram of tissue, we can still only account for 6 μg noradrenaline, or 0·6 % of the quantity infused. Axelrod, Weil-Malherbe & Tomchick (1959) have studied the location of ^{3}H-labelled adrenaline in tissues, following its infusion into the cat. From their data it is apparent that the sympathetically innervated tissues store only a small percentage of the total amount of adrenaline infused, although in whole mice about 30 % of unchanged adrenaline was still present in the

body 20 min after an intravenous injection. Goodall, Kirshner & Rosen (1959) found that in man radioactivity corresponding to 11 % of ^{14}C-labelled noradrenaline appeared in the urine in the first hour after an infusion and 67 % was recovered in 24 hr. They suggest that tissue cells pick up noradrenaline, store it as a complex and gradually release it for metabolism, a conclusion also reached by Axelrod *et al.* (1959). There are at least two possibilities to reconcile this suggestion with the low percentage of infused noradrenaline which we found in tissues. First, that inactive metabolites may also be bound in tissues, as Axelrod *et al.* found for 3-O-methyladrenaline, and secondly, that the complex of noradrenaline in the tissue stores may not be readily split to yield biologically active noradrenaline by the mild extraction procedures we used.

The infusion of dopamine into the cats used in these experiments produced approximately the same response on the blood pressure as the infusion of noradrenaline at 1/25th of the dose. Burn & Rand (1960*b*) have shown in reserpine-treated cats that dopamine infusion will restore the pressor response to tyramine and the dilatation of the pupil. From examination of their figures (Figs. 2 and 6 in Burn & Rand, 1960*b*) it appears that the restoration produced by dopamine is roughly equivalent to that produced by 1/20th of the dose of noradrenaline.

Burn & Rand (1960*b*) showed that L-DOPA infusion restored responses to sympathetic nerve stimulation and to tyramine when these were ineffective after reserpine treatment, and that the restoration was longer lasting than that following a noradrenaline infusion. We found that an infusion of L-DOPA produced a relatively small increase in the noradrenaline content of the tissues, which could serve to restore the response of the tissue, but if in addition the infusion provided a reserve of the precursors of noradrenaline in the tissues available for further synthesis, we can understand why the restoration was longer-lasting than that produced by a noradrenaline infusion.

The infusion of L-DOPA increased the content of noradrenaline in tissues (kidney, uterus, spleen) in 2 out of 8 experiments in intact normal cats and in 6 out of 8 experiments in intact reserpine-treated cats. The smaller uptake observed in noradrenaline content in normal cats may be the result of suppression of synthesis; when the noradrenaline content of the tissue is reduced by reserpine treatment, synthesis may proceed more readily. The smaller increase in noradrenaline content of normal cat's tissue compared with reserpine-treated cats following dopamine infusion can be understood in the same way.

Our results indicate that the abdominal viscera must be intact if the infusion of L-DOPA is to produce a deposition of tissue stores of noradrenaline. Holtz (1959) states that high concentrations of DOPA decarboxylase

are found in kidney, liver, pancreas and intestine. In our evisceration experiments the pancreas and intestines were removed, and the liver was deprived of both its portal and hepatic blood supply. The kidney which remained in the circulation during the infusion was apparently not a sufficiently active source of DOPA decarboxylase to lead to an increase in the noradrenaline content of tissue, nor indeed to convert enough DOPA into a pressor metabolite.

SUMMARY

1. The infusion of 1 mg of noradrenaline into reserpine-treated or untreated eviscerated cats led to an increase in the noradrenaline content of the kidney and uterus.

2. The infusion of 25 mg of dopamine into cats increased the pressor activity of extracts of kidney and uterus on the blood pressure of pithed rats.

3. The lack of response of the uterus of the reserpine-treated cat to hypogastric nerve stimulation was corrected by infusion of dopamine.

4. The infusion of 25 mg of L-DOPA increased the noradrenaline-equivalent of kidney, uterus and spleen of reserpine-treated cats when they were not eviscerated. L-DOPA was pressor in these cats and enhanced the impaired pressor response to tyramine. In eviscerated cats L-DOPA did not increase the noradrenaline equivalent of tissues, was not pressor, and did not enhance the response to tyramine.

We wish to thank Professor R. H. Thorp for providing the facilities and Professor J. H. Burn for his encouraging and enthusiastic suggestions which led us into this work. Dopamine and tyramine were a gift from Dr I. M. Slater of Eli Lilly, Indianapolis; L-DOPA was supplied by Sigma, noradrenaline by Hoechst and reserpine by Ciba.

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SURPLUS EXCITATION IN REFLEX ACTION OF MOTONEURONES AS MEASURED BY RECURRENT INHIBITION

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The antidromic or recurrent inhibition (Renshaw, 1941, 1946) of ventral horn cells was studied in decerebrate cats by Granit, Pascoe & Steg (1957) with tonic discharges to stretch from functionally isolated ventral root filaments belonging to knee extensors. The antidromic shock was applied to a portion of the same root. When triggering the antidromic shock from the discharging spike they found that the frequency of the firing cell often decreased to zero or to a very low value in a cumulative fashion (as here in Fig. 1). Quite often, however, the recurrent inhibition immediately reduced the frequency of discharge to a constant value and thus was non-cumulative. These two modes of behaviour of recurrent inhibition are strikingly different, and failure to understand why sometimes one, sometimes the other effect of recurrent inhibition occurred inspired us to undertake the experiments reported below.

It soon became evident that it was necessary to approach this problem —so essential for the understanding of both recurrent inhibition and the control of discharge frequency of motoneurones—by setting out from a hypothesis with assumptions rigid enough to make it possible to test them experimentally. These assumptions are formulated in the first section of Results. With their aid it has proved possible to develop a method of measuring what we have called 'surplus excitation' in reflex activity of motoneurones. This paper is mainly devoted to the experimental elaboration of this result and the formulation of some useful concepts. In a second paper (Granit, Haase & Rutledge, 1960) we use these concepts in an analysis of the general problem of control of frequency of discharge of motoneurones.

From the work of Eccles, Fatt & Koketsu (1954), confirmed in some essential aspects by Brooks & Wilson (1959) and Wilson (1959), it is known that recurrent inhibition acts by hyperpolarizing the motoneurone cell membrane via the Renshaw cells, which according to Szentágothai (1958)

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also can be identified histologically as small cells lying ventromedially to the motoneurones. These cells discharge spontaneously (Frank & Fuortes, 1956; Curtis & Eccles, 1958) and are more likely to do so in our decerebrate 'tonic' preparations than in the anaesthetized animals of most other authors. Besides the 'tonic' motoneurones are particularly well provided with recurrent collaterals and Renshaw cells (Granit, Pascoe *et al.* 1957; Kuno, 1959; R. M. Eccles, A. Iggo and M. Ito, personal communication) and so their natural tonic activity will maintain 'natural recurrent inhibition' as a permanent hyperpolarizing influence on the motoneurone cell membrane. This effect will increase when the number and rate of firing of the tonic cells increases.

METHODS

Experiments of the kind required for the present work on tonically discharging single motoneurones can succeed only with lively preparations capable of responding in this way. Therefore decerebrate cats have been used. In other respects the technique is essentially as described by Granit, Pascoe *et al.* (1957), i.e. denervation of the leg used with the exception of the medial and lateral gastrocnemius nerves, isolation of the knee extensors (triceps surae) sometimes with separation of gastrocnemius and soleus, laminectomy for operations on the spinal cord, and functional isolation of single ventral root fibres capable of delivering a tonic reflex discharge in response to pull on the muscle, as in Fig. 1*A*. In Arrangement *A* the antidromic shock stimulates a portion of the same root from which the functionally isolated fibre emanates. The tonically discharging fibre was generally localized in the ventral root of L7, which was severed so that the antidromic shock could be delivered to a portion of this root while S1 and L6 were left intact in order to have some γ support of the muscle spindles which elicit the reflex to pull on the muscle (Eldred, Granit & Merton, 1953; Matthews & Rushworth, 1957; Matthews, 1959*a*, *b*). In spite of this precaution it is necessary to keep on stretching the muscle at regular intervals while the experiment is in progress, since the reflex tends to diminish after every prolonged pause.

In most experiments the discharging spike triggered the antidromic shock, as illustrated in Fig. 1*A*: in some, independent iterative stimulation of the ventral root was used. In the former case the stimulus was synchronized with the discharging spike, in the latter it was independent of its rate of firing. Unless recurrent inhibition is strong enough to silence the cell, synchronized stimulation offers the advantage of giving a more regular frequency of discharge to count. The original records below the diagram of Fig. 1 illustrate a control pull on the muscle (record 1) followed by the same pull (record 2) when the antidromic shock was locked to the spike in the manner described. Below each record is a tracing given by the electronic length-recorder used to record extension of the muscles. The 'cumulative' lengthening of discharge intervals with the triggered antidromic shock should be noted (record 2). Generally the spike triggered the shock without delay. A shock delay up to 10 msec was never found to have a significant effect on the recurrent inhibition obtained, suggesting that in a tonic preparation the average increase in the rate of discharge of the Renshaw cells is more important than the duration of firing in response to each individual shock.

Arrangement *A* permitted two further modifications of the experiment: (i) section of the muscle nerves and stimulation of them by repetitive electrical shocks instead of pull; (ii) section of all lumbar and sacral ventral roots.

The purpose of Arrangement *B* (Fig. 1) was to stimulate the cut dorsal roots and use the extensor muscle nerves for the antidromic shock, because this makes the latter influence extensor neurones only. For some problems this is of importance, since Wilson (1959) and

Wilson, Talbot & Diecke (1959, 1960) have found that recurrent stimulation sometimes has reciprocal effects on extensors and flexors. This means that recurrent inhibition from unidentified root filaments can be contaminated by recurrent excitation, even though in the experience of these authors the excitatory effect from flexors on extensors is small, and larger the other way round. The only reason for using Arrangement *B* was to make certain that the

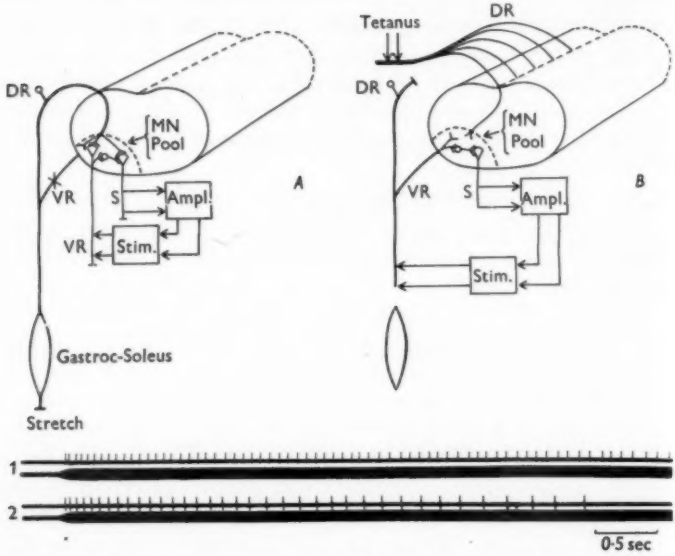

Fig. 1. Experimental arrangements *A* and *B* and sample of experiment with arrangement *A* below. Symbols, DR, dorsal root; VR, ventral root; S, single fibre from ventral root; MN, motoneurone. Arrangement *A*. Spike from single ventral-root fibre amplified and connected to start antidromic shock from stimulator to portion of VR of same segment. Recurrent loop shown. Cross on VR to indicate that in some experiments all lumbosacral ventral roots were severed: such experiments not illustrated in text. Arrangement *B*. Lumbosacral dorsal roots severed and spike in single fibre discharged from DR of its own segment. The amplified spike now triggers shock to the medial and lateral gastrocnemius nerves severed just above muscle. Recurrent path of antidromic impulse shown as in *A*.

Spikes activated by stretch of knee extensors in the manner shown by record 1 below. Record 2 is same strength of pull but with antidromic shock locked to tonically discharging spike (note artifact). Rate and length of pull (15 mm) indicated by length-recorder below.

effects described also occurred with pure recurrent inhibition (between extensor motoneurones of gastrocnemius-soleus). Since with this arrangement all dorsal roots from L4 or L5 downwards are severed, the antidromic shock to the gastrocnemius nerves (see Diagram) can only enter through ventral roots. Arrangement *B* does not afford an easy experiment, because stimulus strength and frequency have to be very carefully adjusted for the maintenance of a tonic discharge. If this required exceptionally strong stimuli, there was too much variation of background from trial to trial.

When delivering the antidromic shock to cut ventral roots it is often necessary to split

root filaments in order to find a portion capable of giving good inhibition on the tonic reflex discharge (Granit, Pascoe *et al.* 1957). Whenever gastrocnemius and soleus were separated, most fibres were found to respond to gastrocnemius pull alone. Soleus fibres, in accordance with previous experience (Granit, 1958) have to be searched for, as, indeed, one would expect from the relative size of the muscles and their fibre supply. Though soleus neurones respond much better to stretch (Denny-Brown, 1929; Granit, 1958), it is possible to activate some gastrocnemius neurones also, particularly if stretch need not be limited to the normal range.

RESULTS

Definition of problem

The individual motoneurones taking part in a maintained soleus stretch reflex tend to fire at a constant rate independent of extension (Denny-Brown, 1929; Granit, 1958). The steady state is upheld by what is called *excitatory drive*. In stretch the excitatory drive comes from the muscle spindles (the nuclear bag or annulospiral endings), directly by monosynaptic paths which have but few (10–20), large end-feet on the motoneurone (according to Szentágothai, 1958), and indirectly over polysynaptic routes. Without support from the latter there will be no maintained stretch reflex. The excitatory drive may be regarded as a barrage of impulses which activate a certain number of synaptic knobs per unit time, thus producing a depolarizing current P_{dep}. Opposed to this influence are inhibitory signals from structures such as Renshaw cells and Golgi tendon organs and also after-hyperpolarization (Brock, Coombs & Eccles, 1953); these generate a repolarizing current P_{pol}. By adding P_{dep} and P_{pol} algebraically one obtains the net depolarizing current, the one thought to determinate the firing rate of the cell. This net current is equivalent to the depolarizing pressure of Phillips (1959); we have simply added a more precise definition of the term.

According to this reasoning, the frequency of discharge (F_n) must be a function of the sum of P_{dep} and P_{pol}. Hence

$$F_n = f(P_{dep} + P_{pol}). \tag{1}$$

F_n in this equation is the only quantity which is directly measurable, but we shall proceed to keep F_n constant and to raise the question whether under such circumstances the right-hand term of (1) is always constant also. In order to test this proposition we inject a constant amount of recurrent inhibition P'_{pol} into a stretch reflex. This means adding to the right-hand member of equation (1) the term P'_{pol}. The experiment consists in testing its effect upon the discharge frequency F_n. The experimental problem is therefore: if the constant rate of firing of a maintained stretch reflex in a motoneurone signifies that the depolarizing pressure is constant, then also a constant P'_{pol} of recurrent inhibition ought to have a constant effect whenever applied. It might be added that, to direct electrical excitation of the motoneurone membrane, F_n is a linear function of P_{dep}

(Barron & Matthews, 1938; K. Frank & M. G. F. Fuortes, personal communication). This question will be considered in our second paper (Granit *et al.* 1960).

Recurrent inhibition into stretch reflex

The simplest approach is to start by testing a stretch reflex at regular intervals with a brief antidromic tetanus, properly adjusted in strength and frequency so as to give the necessary range to the test. We use

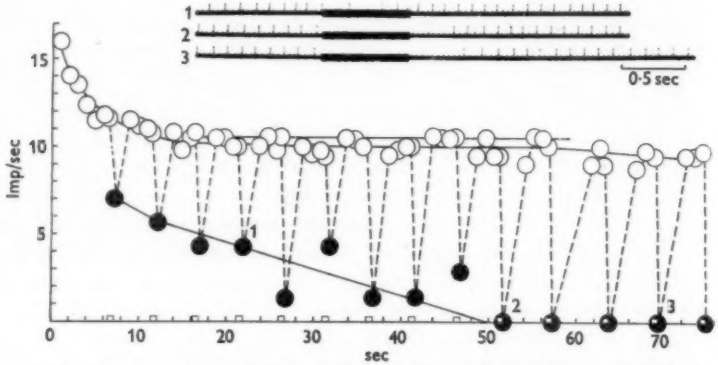

Fig. 2. Arrangement *A* and 15 mm steady pull on the knee extensors. Tetanic antidromic inhibition at 114/sec inserted for 0·7 sec at regular intervals as marked by rectangles on abscissa (running time). Frequency of discharge constant between the two parallel horizontal lines. ●, number of impulses (imp/sec) during the periods of recurrent inhibition. Inset: original records at moments marked 1, 2 and 3 in the diagram. Note that when delayed recovery after recurrent inhibition begins, then discharge frequency fails to reach its original level (at this rate of repetition of antidromic stimulation periods). Discharge stopped for good with last period of stimulation, having been five times temporarily silenced.

Arrangement *A* and pull on the muscle. In Fig. 2 a 0·7 sec antidromic tetanus at 114/sec is injected about every 5 sec. While stretch is being maintained the effect of the antidromic volley is seen to increase, ultimately depressing the discharge to zero. To begin with there was good recovery and even some rebound after each test but in the end, after a number of repetitions of the recurrent stimulus, the discharge frequency started to diminish and ultimately, when the series was completed (shown in the diagram), seven more seconds of waiting for the discharge proved useless. It did not return. It is seen that some variation occurred. The line drawn between filled circles in the diagram serves roughly to indicate the increasing efficacy of recurrent stimulation in spite of constant discharge frequency (between the two horizontal lines). The constant amount of recurrent inhibition P'_{pol} starting from the same basic level of discharge frequency reduces the

latter progressively more and more by an amount that depends upon time after application of stretch. Does this depend on temporal summation of the recurrent tests (P'_{pol}) from moment to moment?

In a good experiment, pull on the muscle can be repeated several times with the same effect and so it becomes possible to lock the antidromic shock to the spike at different moments during the repeated stretch reflexes (as in Fig. 1). Thus recurrent inhibition in each instance is inserted anew into a fresh pull and can be kept locked to the firing spike for any time desired. In such experiments each test with recurrent inhibition is independent and cannot possibly add to the after-effects of a previous test.

In Fig. 3*A* the experiments are of the type just described. There were probably two active fibres though the spikes were of the same size. Measuring began 1 sec after onset of stretch. Attending first to the lines drawn in full, it is seen that in the controls the spike frequency became stabilized at around 40 imp/sec (○). With two identical pulls the antidromic shock (●) was locked early (1) and late (2) in stretch. The very characteristic finding is that suppression to zero took some time in 1 and was practically instantaneous in 2. The interrupted lines refer to conditions to be discussed separately below.

Figure 3*B* refers to another experiment with a different animal. The discharge frequency in steady pull stabilized at 13 imp/sec (illustrated in Fig. 4 with records). The plot is now different. On the abscissa is indicated the moment in pull at which the antidromic stimulus was locked to the discharging spike, on the ordinate the duration required for complete suppression of the discharge. Both curves of Fig. 3*B* show that the later in the discharge the antidromic shock was locked, the more rapid the suppression of the firing. In the original records of Fig. 4 the final phase of suppression is illustrated for curve *B*2 of Fig. 3. The records 3 and 4 serve as controls for 1 and 2, in that they illustrate the discharge frequency at later stages in stretch, as it would have been without early onset of recurrent inhibition by locking the shock to the spike. Curve B1 of Fig. 3 is a result earlier in the day with the same root filament as that used for *B*2. The discharge frequency of the motoneurone was the same as later (*B*2), but recurrent inhibition was more effective.

The general conclusion drawn from such results is that some factor responsible for the maintenance of a constant discharge had spent much of its force in the course of the stretch reflex but that this change had not yet found an expression in the frequency of discharge, which remained constant until tested by antidromic stimulation. This may be restated by defining this factor in a general way as *surplus excitation* with respect to any given discharge frequency, F_n (which by itself gave no sign of the existence of surplus excitation). Thus F_n is stabilized by a frequency-

limiter cutting out the surplus at some point in the system. More attention will be devoted to the problem of frequency limitation in our second paper (Granit *et al.* 1960).

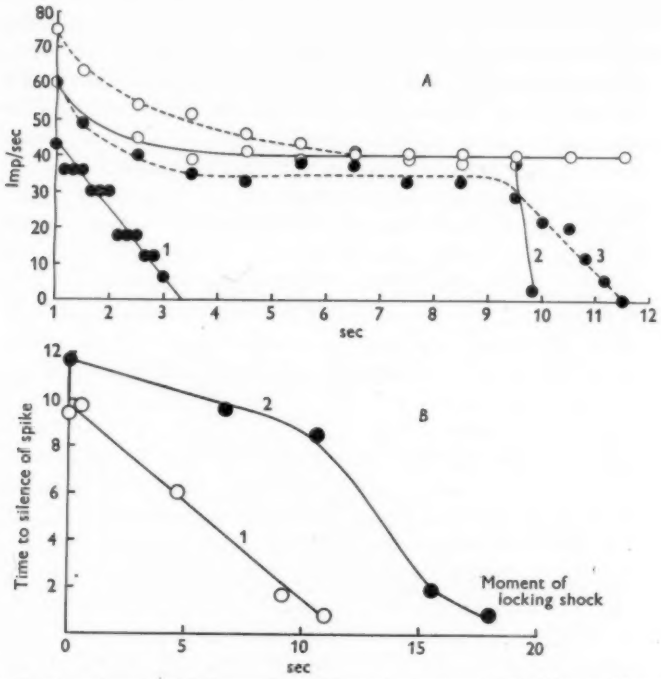

Fig. 3. Experiments with Arrangement *A* varying moment of locking antidromic shock within reflex discharge to steady stretch. Probably two efferent fibres though spikes undistinguishable. *A*. Pulls of 14 mm (lines drawn in full) and 16 mm (broken lines) which between the 6th and 7th sec begin to give the same output frequencies in the controls (○). ●, corresponding pulls with antidromic shock locked to spike from the beginning (1) and 9·5 sec later (2) in different 14 mm pulls, and from the beginning (3) in the 16 mm pull (broken line). In the latter case recurrent inhibition is non-cumulative for 9 sec. After this it suppresses discharge to zero in 2·5 sec. *B*. Another animal. Curves 1 and 2 from two experiments with the same spike, early and later in the day. Its reflex frequency of discharge to steady pull became stabilized at 13 imp/sec to 15 mm stretch in the controls. The time it took to silence this discharge is plotted against the moment at which the antidromic shock was locked. Experiment done with a series of pulls to 15 mm. Sample records of Fig. 1 refer to the same spike and show suppression of discharge to silence.

Now in the case of the stretch reflex we know that surplus excitation diminishes with time, if for no other reason, at any rate on account of the adaptation of the muscle spindles. Yet the output may remain steady for

quite a while. The salient point here is that to a first approximation the amount of surplus excitation may be taken to be proportional to the curves of Fig. 3*B*, i.e. to the time necessary for recurrent inhibition to exert a constant effect, as defined by silence of the cell.

It is important at this stage to draw attention to the experimental fact that in stretch the surplus excitation can actually be destroyed by recurrent inhibition. This change can be traced by studying time for recovery

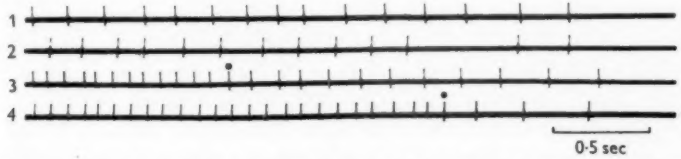

Fig. 4. Original records from which Fig. 3 *B* 2 was plotted. Suppression to zero shown in each case. 1, antidromic shock locked from onset of stretch, time to silence 11·7 sec; 2, corresponding time from moment of locking antidromic shock (10·6 sec) to silence, 8·5 sec; 3, moment 15·5 sec, time to silence 1·9 sec; 4, moment 18·0 sec, time to silence 0·75 sec. Dots mark locking of antidromic shock in 3 and 4.

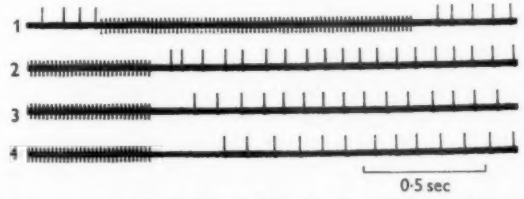

Fig. 5. Same experiment as in Fig. 3 *B* 2, this time with repetitive antidromic stimulation at frequency 63 imp/sec inserted into stretch reflex at moments 11 sec (record 1), 13·4 sec (2), 19·1 sec (3), and 23·9 sec (4). Note tendency to 'doubling' of first pair of spikes after antidromic stimulation and the increasing time of recovery from 2 to 3.

after the tests. In Fig. 2, the inserted records 1–3 refer to corresponding moments in the graph. In them it is seen that time of recovery increased from 1 to 3. The discharging spike used for the graph of Fig. 3*B* (curve 2) was also tested by repetitive antidromic stimuli. The delayed recovery later in stretch, after recurrent inhibition, is well illustrated by the records of Fig. 5. The slightly accelerated recovery (rebound) early in stretch, previously described by Granit, Pascoe *et al.* (1957) as a sign of good excitatory drive, should be noted. It is a common phenomenon and will be taken up below. With tetanic tests, as in Fig. 2, rebound will contribute to the maintenance of the rate of firing between the tests.

Returning to Fig. 3*A*, but this time to consider the interrupted lines alone, the experiment also shows in another way why it is necessary to

conclude that the system possesses a frequency-cut with respect to excitation. We have made use of the fortunate circumstance that maintained soleus stretch reflexes of individual motoneurones may become stabilized at rates of firing which are independent of extension of pull (Denny-Brown, 1929; Granit, 1958, Fig. 1), a fact which in itself demonstrates frequency limitation (for full discussion, see Granit *et al.* 1960). Since for the steady state the input frequency of the afferents from muscle spindles increases linearly with extension (Eldred *et al.* 1953; Granit, 1958) a modest increase of extension (avoiding excess, which will introduce some inhibition) is bound to produce surplus excitation. Now, in Fig. 3*A*, the controls (○) show that ultimately the discharge frequencies become stabilized at much the same value around 40 imp/sec both for the original 14 mm and the second (interrupted lines) 16 mm pull. The curve marked 3 refers to recurrent inhibition, locked from the beginning with the 16 mm pull, and therefore comparable with the one marked 1 for the 14 mm pull. An increase of surplus excitation is demonstrated by the long period of non-cumulative inhibition that now preceded the much delayed drop of rate of firing to zero (cf. also curves 2 and 3). Experiments of this type provided further good evidence for our conclusion that the resistance to recurrent inhibition is determined by the excess of excitation beyond the amount barely capable of sustaining any given rate of firing. The surplus excitation in this case clearly comes from excitatory drive increased by harder pull.

Repetitive and locked antidromic stimulation are compared in Fig. 6, which was chosen also because of the exceptionally strong effect of recurrent inhibition, although in this case the discharge was unusually irregular. The rapid rise of the frequency curve from zero at onset of pull is not included. Antidromic repetitive stimuli at various frequencies (see legend) were compared with locked ones. The effects did not differ very much, in spite of the variations in the mode of applying recurrent inhibition. Yet in experiments with less marked recurrent inhibition the mode of application proved important. The general rule was that if repetitive antidromic stimuli were delivered at rates roughly corresponding to or below the cell's natural rate of firing in the reflex, then synchronized (locked) antidromic shocks were more effective than unsynchronized (cf. Granit, Pascoe *et al.* 1957, p. 398). Otherwise repetition rates from 40 to 50 imp/sec gave stronger recurrent inhibition than did synchronized shocks which were tied to the slow rates of discharge of tonic cells. However, during a tetanus, frequency of discharge tends to be very irregular. It is easier to measure F_n with triggered (synchronized) recurrent inhibition.

Figure 6 also serves to emphasize that, in spite of maintained stimulation by an amount of extension which in the controls was fully capable of keeping up frequency of firing for minutes in the fashion shown, insertion

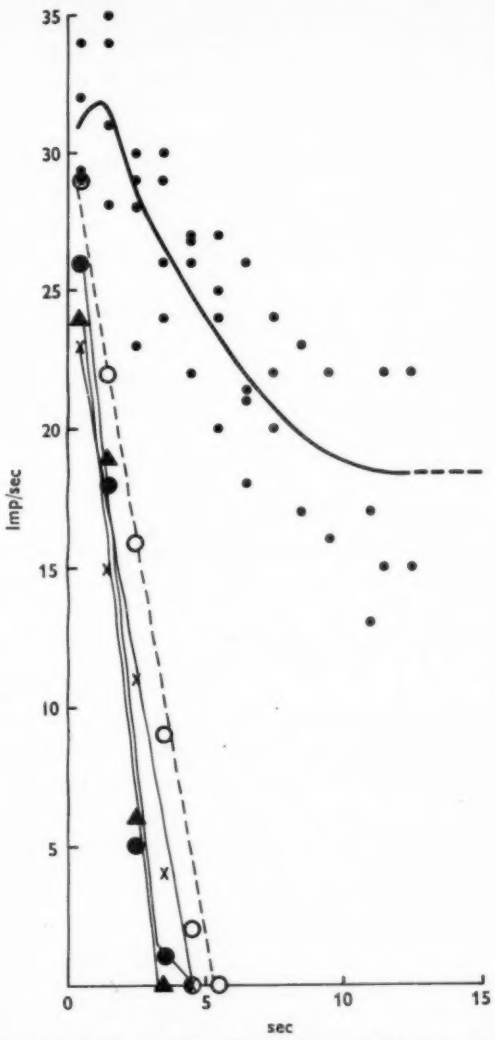

Fig. 6. Arrangement *A*. Control discharge to 14 mm pull (uppermost, small dots) was well maintained as long as pull lasted but irregular, possibly owing to the presence of two indistinguishable spikes. Chosen because of the very powerful recurrent inhibition from onset of stretch. Locked and repetitive antidromic stimulation compared, both maintained until spike was silenced. ○, Locked; ●, rate 14 imp/sec; ▲, 24 imp/sec; ×, 64 imp/sec.

of recurrent inhibition sufficed to do away with the discharge in 5 sec, and very thoroughly at that, since it did not return for the 3 sec the camera was kept running. An amount of recurrent inhibition which is constant (or even diminishing owing to its own effect on rate of discharge) therefore is capable of removing surplus excitation.

To sum up: (i) Somewhere in the discharging system there exists a frequency limiter. (ii) As a consequence any available surplus of excitation is prevented from augmenting frequency of discharge, F_n. (iii) By adding recurrent inhibition it is possible, within limits, in such cases to estimate the amount of surplus excitation by using a constant index, in our case suppression to silence (Fig. 3). (iv) When any given F_n is upheld by a sufficiently small surplus of excitation, recurrent inhibition acts as if it were capable of destroying it altogether with consequent fall of depolarizing pressure to a value from which firing fails to recover. (v) With maintained stretch a progressive loss of surplus excitation is concealed behind a 'screen' of steady frequency of discharge, but its existence can be revealed by testing with recurrent inhibition.

Electrical stimulation of afferents

It is clear that whatever the nature of the function defined by equation (1), a decrease of depolarizing pressure can be produced by adding to the inhibitory quantity P_{pol} of the right-hand member, e.g. by steady pull on an antagonist flexor muscle during electrical stimulation of the extensor afferents. When pull is hard enough, frequency of discharge of the extensor motoneurones goes down, even when a constant electrical tetanus of the severed extensor afferents (efferent roots being cut) maintains a constant drive. The experimental question is therefore: is it possible, with maintained drive, to reduce depolarizing pressure without making non-cumulative recurrent inhibition cumulative, i.e. without silencing the discharge of the cell? It is, of course, necessary to have a good recurrent inhibition to begin with.

The experiment of Fig. 7 has been designed as an answer to this question. The upper record is the control followed by locking of antidromic stimulation, the lower one the corresponding experiment during maintained stretch of the flexor tibialis anterior. There were in all 130 periods of measurement, 1 sec each, pull on flexor and no pull alternating. Control frequency of discharge without pull was $32 \pm 1{\cdot}6$ imp/sec. It was reduced by non-cumulative recurrent inhibition to $18 \pm 1{\cdot}2$ imp/sec. The corresponding values during flexor pull were $25 \pm 1{\cdot}7$ and $13 \pm 0{\cdot}8$ imp/sec. The suppression ratios (control frequency:inhibited frequency) with and without pull were therefore 1·8 and 1·9 respectively, hence equal within the limits of error. Thus, in spite of a definite reduction of depolarizing

pressure, the cell could not be silenced by recurrent inhibition as long as a constant excitatory drive was maintained. A good amount of surplus excitation relative to the value of F_n was thereby maintained. The recurrent inhibition in the present case was very potent, causing a reduction of depolarizing pressure which in terms of frequency of discharge amounted to 45 %. The orthodromic inhibition had reduced depolarizing pressure by an amount corresponding to 7 imp/sec. Thus this type of experiment (of which there were four in all) is an important supplement to those on stretch, in which, however, a slight drop of depolarizing pressure (frequency) was tantamount to loss of surplus excitation with consequent

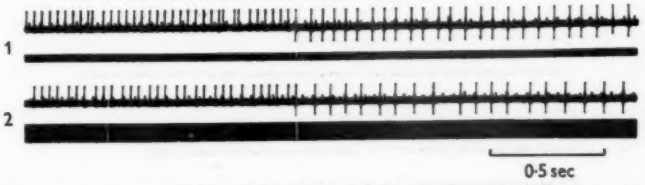

Fig. 7. Arrangement *A*, but electrical stimulation from severed medial and lateral gastrocnemius nerves at 64 imp/sec instead of pull. Nerve to antagonist tibialis anterior preserved intact for 13 mm inhibitory pull in record 2 (see length-recorder below). 1, frequency of discharge 32 imp/sec, suppressed by non-cumulative recurrent inhibition to 18 imp/sec; 2, during pull on tibialis anterior frequency of discharge diminishes to 25 imp/sec and recurrent inhibition now suppresses it to 13 imp/sec. These values refer to averages of measurements for in all 130 periods, each of 1 sec, with and without pull. Contralateral dorsal lumbosacral roots cut in this experiment.

delayed recovery after recurrent inhibition. The experiments on stretch thus differed from the present one in that any fall in the steady rate of firing led to an apparent increase of potency of recurrent inhibition, generally ending in silence of the discharge.

It now remains to use Arrangement *B* in order to have some experiments in which extensor reflex spikes are tested with recurrent inhibition limited to extensor fibres (see Methods). These experiments were restricted to constant discharge frequencies and it was attempted to make use of the frequency-limiter in the system by varying stimulus strength so as to approach the minimum excitatory drive necessary for maintaining a constant discharge frequency.

In Fig. 8 the records 1*a* and 1*b* are continuous and it is seen that recurrent inhibition was non-cumulative. Reduction of stimulus strength in record 2 with little effect on average rate of discharge immediately made inhibition cumulative. In *D*, the records 1*a* and 1*b* refer to the same run. Antidromic stimulation triggered at moments 1·3 sec (1*a*) and 8·7 sec (1*b*) after initiation of repetitive stimulation of the dorsal roots gave

non-cumulative inhibition in the first instance and cumulative inhibition in the second when the discharge had been maintained for a longer time (cf. the counterparts in Fig. 2 with stretch reflexes, first section). Stimulus strength was then slightly reduced without significantly influencing frequency of discharge (= depolarizing pressure). Record *D*2 is the control; record *D*3 shows that recurrent inhibition now was cumulative.

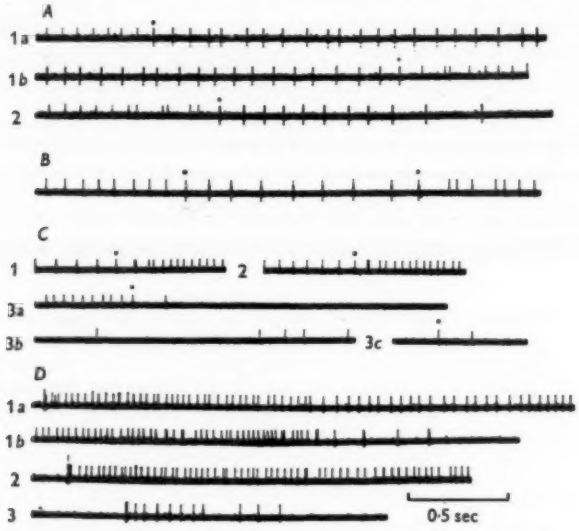

Fig. 8. Arrangement *B*. *A–D* from different experiments. In *A–C* locking and unlocking of antidromic shock marked by dots. Locking identified in *D* by shock artifacts. *A*. 1*a*, directly continued in 1*b*, illustrates non-cumulative recurrent inhibition which by slight decrease of stimulus strength in 2, turns cumulative. *B*. Non-cumulative recurrent inhibition on and off to show tendency to doubling of spikes at cessation of antidromic stimulation. *C*. 1 and 2 show similar doubling of discharge after cessation of locked antidromic stimulation. 3*a–c*, same experiment after reduction of stimulus strength to reduce afferent excitation. 0·5 sec cut out between *a* and *b*; *c*, 0·8 sec after last spike in *b*. Spike kept locked to stimulus until dot in 3*c*. Note in this case that recovery is delayed and feeble, and no doubling in 3*c*. *D*. 1*a* recurrent inhibition non-cumulative when inserted 1·3 sec after initiation of DR stimulation; 1*b* shock, locked to spike in the same stimulation period at moment 8·7 sec from initiation of dorsal-root driving of spike. Recurrent inhibition now cumulative; 2, control after slight reduction of stimulus strength; 3, same stimulus with antidromic shock locked to spike from the beginning.

Like the previous experiments with stretch, these, too, show that with a sufficiently potent recurrent system there is no fundamental difference between cumulative and non-cumulative recurrent inhibition (which are purely descriptive terms). It is all a matter of whether any given moto-

neurone is provided with enough surplus excitation to be able to recover from antidromic inhibition. The experiments also show that in spite of electrical stimulation surplus excitation tends to diminish with continued stimulation. In this respect animals differ very much, as do individual cells in one and the same animal. (This finding has been studied in greater detail by Granit *et al.* 1960).

Of particular interest are the results of records *B* and *C* 1 and 2 (Fig. 8) which show that 'doubling' of spikes (rebound) also occurs with extensor spikes tested by an extensor recurrent inhibition which therefore is

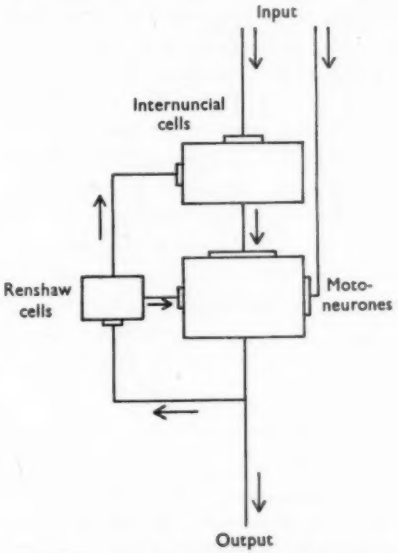

Fig. 9. Block diagram to show recurrent circuit (see text).

contaminated by excitatory effects from flexors (cf. Wilson, 1959; Wilson *et al.* 1959; and Methods). Weakening stimulus strength, as in records 3*a* and 3*b* of Fig. 8*C*, so as to reduce surplus excitation, removed 'doubling'. The results are therefore in line with the interpretation of Granit, Pascoe *et al.* (1957), according to which doubling is due to rebound in the presence of good excitatory drive, rather than with that of Wilson & Talbot (1960) who regard the phenomenon as a genuine excitatory component of recurrent inhibition.

Sometimes, when a powerful recurrent inhibition was combined with a good excitatory drive, one could, when the antidromic shock was locked to the spike, find inhibition cumulate towards a low value for the rate of discharge without ever being able wholly to silence firing. The long pauses

between the spikes allow time for some recovery from recurrent inhibition, and so the firing rate of the spike increases anew. This, in turn, increases repetition rate of the locked antidromic shocks and so cumulation of inhibition is reinstated. In this manner, then, the circuit closed artificially by locking stimulus to spike could go on hunting for its balance point. Such cases are rare compared with those in which the antidromic shocks sum their effect to give a steady or but slightly fluctuating reduction of firing frequency.

DISCUSSION

A block diagram of the possible circuits we have been studying is given in Fig. 9. We regard the connexion recurrent collateral–Renshaw cell–motoneurone to be established (Renshaw, 1941, 1946; Eccles *et al.* 1954; Brooks & Wilson, 1959; Wilson, 1959). The second circuit from Renshaw cells to internuncial cells may start from the recurrent collaterals themselves, though we have drawn it so as to suggest that (some) Renshaw cells may inhibit internuncial cells. Frank & Fuortes (1956) have shown by direct recording from interneurones farther inside the spinal cord that antidromic shocks may elicit discharges also at this site (cf. also Koizumi, Ushiyama & Brooks, 1959). This is in accordance with the original observations of Golgi (1903) who saw recurrent collaterals disappear into the cord. The results of Frank & Fuortes make direct internuncial effects from antidromic stimulation a reality but do not show if such recurrent branches are common or rare.

There is definite evidence for the conclusion that an internuncial excitatory path is of importance for the stretch reflex. Thus, for instance, it was proved by Granit, Phillips, Skoglund & Steg (1957) that the potentiating effect of repeated brief stretches on tonic discharges of extensor motoneurones likewise was potentiated by two polysynaptic reflexes, 'pinna' and 'crossed extensor'. It is also well known from the early work of the Sherrington school that extensor stretch reflexes disappear in acute spinalization, a fact confirmed innumerable times (cf. Alvord & Fuortes, 1953). Internuncial cells therefore contribute to the excitatory drive and the depolarizing pressure. In fact, it is doubtful whether any normal tonic discharge to stretch is ever possible without a substantial fraction of internuncial excitatory support. Pompeiano (1960) has recently devoted a study to the effect of internuncial 'release' phenomena on the stretch reflex.

On activating the input (Fig. 9) at increasing strength in the presence of sufficient internuncial support, the point is soon reached when the discharge frequency of motoneurones becomes practically constant. Thus a surplus of excitation is obtained. This may be regarded as an available reserve just above or a great deal above what is needed to maintain a certain firing frequency F_n. It proved possible in the experiments on stretch to measure this surplus by the time it took to silence the cell under recurrent inhibition. We have been able to show that excitatory drive (which determines surplus excitation) in some types of experiment is

destroyed by recurrent inhibition. We have also found that reduction of depolarizing pressure by itself (by flexor pull creating inhibition) need not necessarily augment the effect of recurrent inhibition, provided that the excitatory drive is well maintained by electrical stimulation. In our second paper (Granit *et al.* 1960) we discuss frequency limitation and depolarizing pressure. For the time being it is concluded that the polarizing effect of recurrent inhibition can balance out with depolarizing pressure as a steady (non-cumulative) state only when there is enough surplus excitation available. This comes from presynaptic excitatory drive. 'Cumulative' and 'non-cumulative' recurrent inhibition are merely descriptive terms. Non-cumulative inhibition turns cumulative when surplus excitation barely suffices to maintain any given frequency of discharge.

From the work of Eccles *et al.* (1954) it is known that recurrent inhibition has a hyperpolarizing action at the motoneurone membrane. It will thereby oppose depolarization. Thus it will reduce depolarizing pressure (see diagram of Fig. 9). Whatever form we assume for equation 1 (which is likely to be linear, as discussed in our second paper, Granit *et al.* 1960) it is clear that aur results show that the net effect of recurrent inhibition on F_n cannot be deduced from depolarizing pressure without taking into account excitatory drive. This is at least partly, and probably largely, a presynaptic influence maintaining P_{dep}, and is not itself included in the terms of equation (1). The excitatory drive, on this view, determines the rate at which the motoneurone cell membrane can compensate by depolarizing currents for the hyperpolarizing effects of recurrent inhibition.

The finding that recurrent inhibition can under some circumstances be used as an instrument for gauging the available amount of surplus excitation by which in reflex action any given depolarizing pressure is maintained, is one we find a little difficult (though not impossible) to understand on the basis of a recurrent inhibition solely directed towards the motoneurone membrane. The experiments presented have shown with considerable uniformity, and particularly clearly with natural stretch reflexes, that at times recurrent inhibition destroys excitatory drive and silences the motoneurone for good in spite of maintained stretch. We must therefore seriously consider the hypothesis that the recurrent process also destroys it at its source in the interneurones, acting, say, in the manner of a temporary acute spinalization. This process is often heralded by signs of delayed recovery after recurrent inhibition and ends in complete failure of the discharge to reappear, though the latter is well enough maintained in control pulls run for the same or greater lengths of time.

It has been pointed out by Matthews (1959*a*, *b*) that stretch reflexes for any given extension produce the same amount of static reflex tension independently of whether they are started by very slow or by fast pull.

Hence it is difficult to understand why a muscle, kept extended, could not reproduce its reflex tension after temporary recurrent inhibition (Figs. 2, 3*a*, 4 and 5), assuming the latter to be directed merely on to the motoneurone cell membrane. To explain on the basis of the findings of Eccles *et al.* (1954) the fact that it cannot do so, it would be necessary to add the assumption that a high afferent starting frequency is necessary for activating the internuncial pool. Alternative hypotheses on similar lines could be formulated to account for the failure of a cell, blocked by recurrent inhibition, to regain its active state. Thus, Granit, Pascoe *et al.* (1957) suggested that post-tetanic potentiation at the synapse between Renshaw cell and motoneurone might strengthen the effect of recurrent inhibition by temporal summation. There is also accommodation to consider (Araki & Otani, 1959) and a firing cell, as we have seen, is supported by rebound.

Be this as it may, with regard to the physiological problems of regulation of rate of firing and general control the essential point is that recurrent inhibition—when well developed anatomically—powerfully influences those particular motoneurones that are kept firing on too small a supply of surplus excitation. The actual frequency of discharge of the motoneurone is immaterial. If barely maintained, recurrent inhibition will stop the firing. Thus it can be understood why Phillips (1959) often found what was interpreted as recurrent inhibition in the pyramidal cells with high firing frequencies, whereas Granit, Pascoe *et al.* (1957), found it to be strong in tonic ventral horn cells which fire slowly (cf. Kuno, 1959; R. M. Eccles, A. Iggo and M. Ito, personal communication). We have often seen high-threshold, rapidly firing cells silenced before low-threshold tonic ones run on a greater surplus of excitation. If with electrical stimulation spikes were kept actively discharging just at threshold strength, then, whatever their rate of firing, they tended to disappear as soon as antidromic stimulation was triggered to the spike, provided that they had recurrent inhibition. This was well seen whenever functional isolation of ventral root filaments for single spikes was unsatisfactory so that high threshold neurones were seen to be brought in at increased stimulus strength. In this case the feebly supported high-threshold repetitive discharges were usually silenced by recurrent inhibition.

One definite role of recurrent inhibition will therefore be to hold down the subliminal and just liminal fringe around any reflex action and to stop feebly supported discharges from lingering on. Since its effect depends upon how well any particular depolarizing pressure on a motoneurone is supported by surplus excitation, different forms of physiological activity may vary very much in this respect. Inasmuch as organizational features are important for recurrent inhibition, they will determine its effect in accordance with the principle now established.

Granit, Pascoe *et al.* (1957) regarded the recurrent collaterals as the 'natural efferent antagonists to the γ-driven tonic system' (p. 397) meaning that, since spindle activation was slow and was succeeded by long-lasting states of post-tetanic potentiation, a 'danger zone' of lingering effects was created which, in the end, was neutralized by recurrent inhibition. This deduction is included within, and not an exception to the generalization of the present work as given in the previous paragraph. They also noted that (what has been called here) surplus excitation, as obtained by post-tetanic potentiation from the muscle afferents, made cumulative recurrent inhibition non-cumulative, in fact, the urge to formulate and study the present problems came from their observations. Eccles *et al.* (1954) held recurrent inhibition to be a kind of safeguard in violent motor activity, a role which it could play well only with motoneurones from the subliminal fringe.

Wilson (1959) and Wilson *et al.* (1959) have found recurrent inhibition coupled with reciprocal excitation. This is evidence for its role in functional differentiation of reflex patterns, but further work will have to decide how strictly it follows the principle of reciprocal action. As a matter of fact, R. M. Eccles, A. Iggo and M. Ito (personal communication) did not find reciprocity to be the decisive organizational feature, but after testing with very many different nerves decided upon 'proximity' in the cord. The excitatory effect we have not seen with tonically discharging extensor motoneurones and so recurrent inhibition must be vastly more potent in them. Also in these workers' results by the intracellular technique excitatory effects were rare and very small. Brooks & Wilson (1959) and Brooks (1959) concluded from the greater effect of recurrent inhibition on heteronymous than on homonymous reflexes that it served to concentrate the reflex field, acting much like Hartline's lateral inhibition (Hartline, 1949; Hartline & Ratliff, 1956). This deduction also falls under the generalization of the present work as a special case. Whenever there is a discrepancy between excitation actually used and excitation necessary to run any particular reflex against recurrent inhibition, the result can be predicted in its main outlines from this generalization. However, we show in our second paper (Granit *et al.* 1960) that the Renshaw cells are under central control and so, in the end, the scope of their influence will ultimately be determined by the extent to which they are mobilized.

SUMMARY

1. Tonic reflexes in single motoneurones of decerebrate cats have been elicited by extensor stretch and by electrical stimulation of muscular afferents or dorsal roots and tested by antidromic stimulation at constant strength.

2. When care is taken with steady stretch to maintain a steady reflex frequency of discharge, the effect of recurrent inhibition of constant strength and duration nevertheless increases, when it is injected later and later in the maintained reflex discharge. This is shown to signify that the steady discharge is kept up by a slowly diminishing surplus of excitation, not visible in the rate of firing, but gauged in this experiment by the relative increase in efficacy of recurrent inhibition. Hence motoneurones are provided with a frequency-limiter (cf. Granit *et al.* 1960).

3. By making use of the frequency-limiter to obtain stretch reflexes at the same rates of discharge in spite of different amounts of excitatory input, it is shown that recurrent inhibition actually remains constant or increases but slowly in potency, as long as there is evidence for surplus excitation on the input side. A cumulative or increasing effect of recurrent inhibition, under various conditions, is always found to signify that the steady rate of discharge of the cell is inadequately supported by the prevailing excitatory input.

4. Adequate support of any reflex discharge can be obtained with electrical stimulation of afferents. If under such circumstances the tonic extensor reflex is partially inhibited by steady pull on the antagonist flexor so as to reduce firing rate, this need not augment recurrent inhibition as does a negligibly small reduction in firing rate due to loss of surplus excitation. Reduction in motoneurone frequency of discharge is not as such decisive, but rather the amount of presynaptic excitatory drive by which any discharge is supported.

5. One major physiological role of recurrent inhibition is therefore to suppress feebly supported motoneurone activity from the 'fringe' and to prevent after-discharges from lingering on.

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RECURRENT INHIBITION IN RELATION TO FREQUENCY OF FIRING AND LIMITATION OF DISCHARGE RATE OF EXTENSOR MOTONEURONES

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Our previous paper (Granit & Rutledge, 1960) showed that recurrent inhibition silences the discharge of a motoneurone which is feebly supported by excitatory drive, even in the face of constant depolarizing pressure as defined in that work. One answer was also provided thereby to the general question of why sense organs and interneurones fire at frequencies which are in excess of immediate apparent needs. In the present study every precaution was taken to maintain afferent excitation in excess of that barely needed to keep up a given rate of reflex firing to electrical stimulation of muscular afferents. This is because the main question here concerns the relationship between normal firing frequency F_n of a motoneurone and its rate of discharge F_i under recurrent inhibition. F_i was now found to be a basically linear function of F_n. This finding proved to be of methodological interest in work on the physiological significance of recurrent inhibition.

Holmgren & Merton (1954), making use of an analogy derived from electronics, suggested that recurrent inhibition has a stabilizing effect on the discharge from motoneurones. Defining stabilization for our purpose as the integrated net effect of one or several processes engaged in holding the rate of firing within relatively narrow limits, we have also tried below to scrutinize this concept as a biological proposition. (It is not our intention to elaborate an electronic analogy which may or may not be valid.) This means that special attention will be given to the factors which determine the upper and lower limits of the frequency range. Thus limitation of discharge frequency is possibly a more accurate term than 'stabilization'.

We are not aware of any previous work concerned with the relation between F_n and F_i. The general problem of 'frequency limitation' may,

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however, be said to have arisen out of Adrian & Bronk's (1929) and Denny-Brown's (1929) work on the reflex activity of single motoneurones whose firing they proved to be restricted to fairly low rates. Out of the many papers confirming this finding, attention may be drawn to those of Alvord & Fuortes (1953), Lloyd (1957), and Margaria (1959) as being of interest for the present work. The question by what means the discharge frequency of motoneurones is limited (upwards) has not really come into the foreground until fairly recently. Further relevant contributions will be found in papers by Granit, Pascoe & Steg (1957); Eccles, Eccles & Lundberg (1958); Granit (1958); Matthews (1959*a*, *b*); Phillips (1959); Granit & Rutledge (1960) and Pompeiano (1960).

METHODS

Nothing technically new is introduced in this work. Arrangement *A* of the previous paper (Granit & Rutledge, 1960) was used. The precollicular decerebrate animals were deefferented, generally from L 6 to end of cord but always within the segments used. Stimulating electrodes were placed on the severed central stumps of the medial and lateral gastrocnemius nerves, tetanic rates of not less than 114/sec, rarely more, being used to elicit tonic firing from functionally isolated motoneurones in L 7 or S 1. In agreement with Alvord & Fuortes (1953) we think this a good way of setting up a central excitatory state, in the original sense of Sherrington. By chance our standard rate of stimulation fell near the optimal values (70–100/sec) which, while this paper was in preparation, were published by Curtis & Eccles (1960) for monosynaptic post-synaptic potentials in motoneurones. The isolated reflex spikes from one ventral root filament were made to trigger the antidromic shock to the rest of the root, as is described in the previous paper (its Fig. 1) and also illustrated with records from an original experiment in Fig. 1 of this paper.

The standard procedure consisted in measuring the frequency of discharge, F_n, during 5 sec of control, its rate, F_1, during 5 sec of locked antidromic stimulation, and finally F_n in recovery for 5 sec afterwards, the last in order to have a check on possible loss of drive. The two control values for F_n, before and after antidromic stimulation, were averaged. For special problems shorter times were used. This averaging means including a component of rebound (Granit, Pascoe *et al.* 1957) but since this process may have started already during stimulation it is safer to use two averaged control values. Different frequencies of discharge F_n were obtained by variation of stimulus strength and it was attempted to find motoneurones that could be made to vary in rate of discharge by these means. The time of onset of antidromic relative to onset of afferent stimulation was constant for any one series of observations at different frequencies, unless the effect of afferent stimulus duration had been specifically proved to be insignificant for the particular neurone used. This will be separately discussed below. The most common times for onset of antidromic stimulation fell between 20 and 30 sec after initiation of afferent stimulation.

In every case the isolated spike was tested from threshold or minimum frequency for tonic firing, F_n min., to maximum obtainable, F_n max., by increasing afferent stimulus strength. Stretch afferents excite a large number of interneurones (Kolmodin, 1957) and that interneurones actually contribute to reflex excitation of extensor motoneurones has been proved by Granit, Phillips, Skoglund & Steg (1957). The excitatory state thus obtained should not be confused with monosynaptic effects on motoneurones. The decerebrate animal is in a state of extensor release and flexor suppression, as is known from the work of the Sherrington school and recently elaborated by Job (1953), Eccles & Lundberg (1959) and Holmqvist & Lundberg (1959). Afferent stimulation of the extensor muscle nerves influences

this 'state' and does not specialize on monosynaptic effects, as was clearly realized by Alvord & Fuortes (1953).

Locking of the antidromic shock to the discharging spike ensures that the antidromic inhibition is forced to follow the dominant rhythm F_n of the spike studied. The rhythm F_i will emerge as a balanced state between orthodromic depolarization and recurrent (re)polarization (Eccles, Fatt & Koketsu, 1954) of the motoneurone membrane, evaluated as frequency of discharge.

RESULTS

Relation between F_n and F_i

At the outset it is necessary to realize that the experiment deals with a complex situation. When stimulus strength is augmented in order to increase F_n, several other motoneurones are excited in parallel with the one studied and many of them will be provided with Golgi recurrent collaterals. Thus, to an unknown degree, 'natural' recurrent inhibition

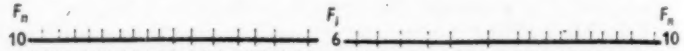

Fig. 1. Records from three experiments showing tonic reflex discharge of single fibre in ventral root to afferent stimulation at repetition rate 114/sec. F_n is normal frequency of discharge 1 sec before and after locking of antidromic shock to firing spike to obtain F_i. Values of F_n and F_i (impulses/sec) against the records refer to the cut out portions and not to total period of counting. The first five rows, from above downwards, refer to one experiment, the sixth and seventh to two other experiments; the seventh is put in to illustrate good rebound.

will compete with the experimentally injected component for the available number of Renshaw cells, and some rivalry will occur up to the saturation point (defined as the maximum number of maximally active Renshaw cells). More afferent inhibition will also be introduced by an increase of stimulus strength, but this need not concern us in the present work for which it is important merely to have a semistationary state of balance between excitatory and inhibitory forces, with consequent steady rate F_n of firing. It is immaterial what depolarizing and polarizing forces participate in building up the net depolarizing current—defined as depolarizing pressure ($P_{dep}+P_{pol}$)—as long as excess drive is present, and this should be obtainable by electrical stimulation above the threshold (for definitions, see Granit & Rutledge, 1960).

Figure 1 serves to introduce the actual experiment. The first five hori-

zontal rows are from one cat, the two lowermost from two other animals. In each row the last few control values before antidromic stimulation are shown on the left, then the initial portion of the 5 sec antidromic stimulation, the last portion of it, and finally the control afterwards. Strictly, 5 sec of each portion should be illustrated to give the full experiment with its greater accuracy. The figures indicating discharge rates in the records refer merely to the strips cut out for reproduction. The rebound in the lower records should be noted.

The curves *A* to *G* and *I* of Fig. 2 are from motoneurones isolated in one single animal; *H* is from another animal. The data have been plotted in terms of F_i as ordinates, against F_n as abscissae. Straight lines have been drawn through the points. Curve *H* was added to show how nearly the lower limit can be balanced: at $F_n = 15$/sec recurrent inhibition silenced the cell because it was difficult at the threshold to mobilize enough surplus excitation (see Granit & Rutledge, 1960). This value corresponds to F_n min. After a slight increase of stimulus strength the discharge rate went up to $F_n = 16$/sec, at which level a balanced discharge was obtained and thereby the first point on the curve. The many values, heaped around the top of several curves, bear witness to fruitless attempts to increase F_n max. by augmenting shock strength to the afferent nerve. Curve *I* is an interesting special case in which the linear relation between F_n and F_i was valid in spite of the fact that two different spikes were present and, possibly, three in the end. Complications are common in such cases. For instance, at a certain rate of discharge one of the two may actually be facilitated by the rebound of its predecessor and so, quite suddenly, adopt its rate of discharge. For this and other similar reasons all our analyses are based on single-fibre preparations. The results may then be conveniently summarized by a linear equation for which we have preferred the less common form

$$F_n = aF_i + b. \qquad (1)$$

The values of the constants a and b will be found in the legend, b being the intercept for $F_i = 0$. Put in this form, a increases when recurrent inhibition increases and more often than not b is positive. The constants have been derived from curves drawn freehand, as no particular purpose was served by calculating the ideal straight lines.

The efficacy of recurrent inhibition is given by the difference between the normal and the inhibited rate of firing, which is wanted as a function of F_n and easily derived from equation 1. It is

$$F_n - F_i = F_n(a-1)/a + b/a, \qquad (2)$$

from which it emerges that the plot of equation (1) is useful also because it represents a convenient way of deriving the values of the constants of equation (2). This, in fact, has been our procedure and in the present case

the curves of Fig. 2 have been replotted in Fig. 3 for $F_n - F_i$ as a function of F_n in accordance with equation 2. The simple formulas used should not be interpreted as a mathematical treatment of recurrent inhibition. Their

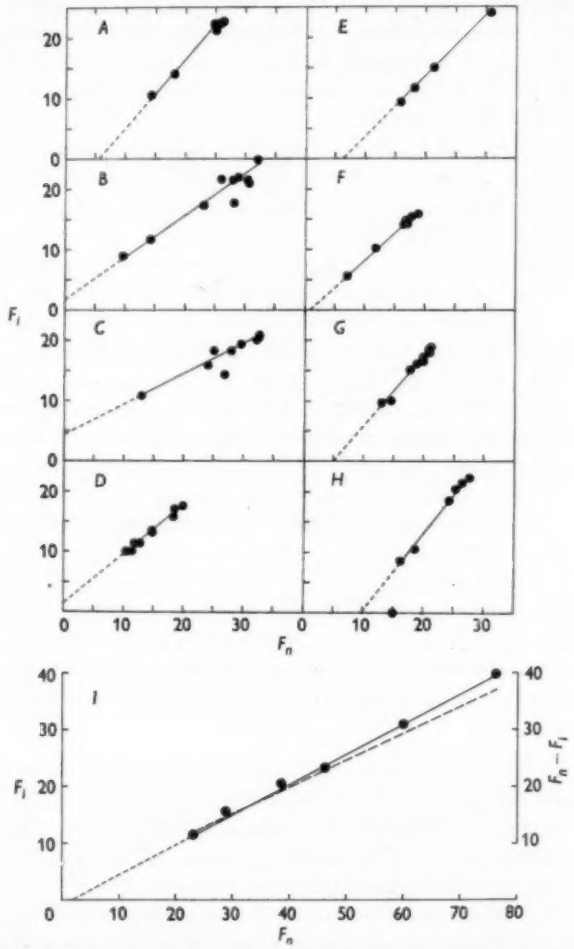

Fig. 2. Plot of F_i as ordinates against F_n as abscissae (imp/sec) for a number of isolated motoneurones *A–G* and for a pair of motoneurones *I*, all from single experiment, *H* being from another animal and put in to show the critical effect of lack of surplus excitation, as explained in text. Curves drawn free-hand to equation $F_n = aF_i + b$. The values of the pairs a and b from *A* to *H* are 0·96, 4·3; 1·47, −3·0; 2·01, −9·2; 1·22, −1·8; 1·00, 6·3; 1·10, 1·0; 0·86, 5·0; 0·79, 9·8. Ordinate scale on the right for curve *I* and interrupted curve refers to plot of $F_n - F_i$ against F_n as in next figure.

purpose is merely to simplify and concentrate description of our findings to essentials.

When we focus attention on the derived constant $(a-1)/a$, it is seen that if $a = 1$, the amount of recurrent inhibition is wholly determined by b, and the curve (in the plot of Fig. 3) is a horizontal line at ordinate b, as in E of that figure. Recurrent inhibition then is constant and independent of rate of discharge F_n. If $a > 1$, then $F_n - F_1$ increases in proportion to the normal discharge frequency F_n, as shown by curves B, C, D and F of

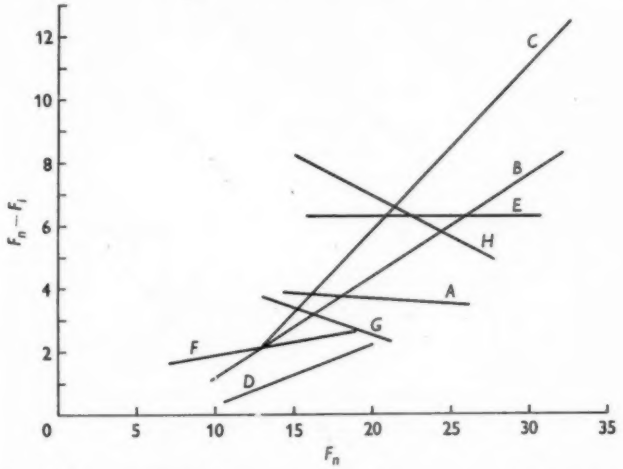

Fig. 3. Plot of curves derived from those of previous figure and similarly marked but using equation 2 of text. Ordinates, $F_n - F_1$; abscissae, F_n (imp/sec.). Each curve also shows the experimentally obtained range from F_n min. to F_n max.

Fig. 3. Finally, if $a < 1$, $F_n - F_1$ diminishes with F_n, as illustrated in A, H and G. Thus in this experiment all possibilities were sampled, though it is more common to find one particular animal to some extent specializing, as it were, on positive or negative slopes $(a-1)/a$.

As has been stated, all values for F_n were brought to the maximum obtainable by electrical stimulation of the muscle nerve. When the range within which F_n could be made to vary was very small, below 6 imp/sec, we have not included the data in our survey of Fig. 5, because in such cases the question of linearity hardly arises. Actually small ranges were quite common, but being interested in the relation between F_n and F_1 we have naturally tried to select spikes capable of varying in frequency of discharge. In order to demonstrate that linear curves do provide a good description of our findings it is of interest to reproduce seven curves ($A-G$ of Fig. 4) comprising large ranges in addition to the two (B and C)

already shown in Fig. 2. Occasional single values may fall outside but we shall provide evidence below for the conclusion that recurrent inhibition is under supraspinal control, and decerebrate animals are well known to have occasional fits of activity.

Sometimes recurrent inhibition has a threshold above the minimum maintained value of F_n, but generally F_n and recurrent inhibition start

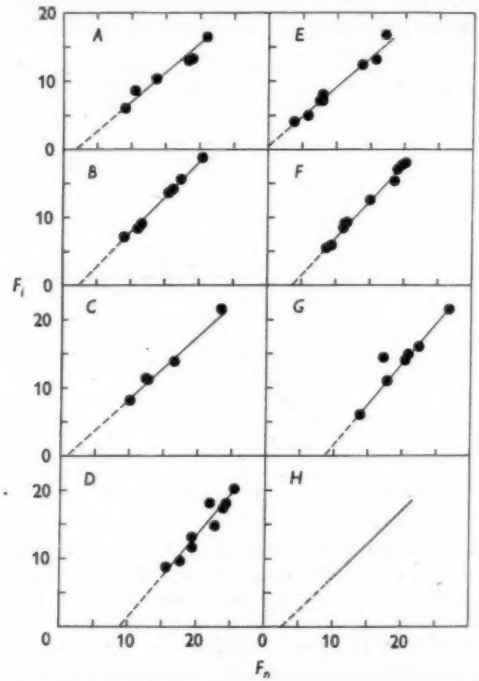

Fig. 4. *A–G* are curves plotted as in Fig. 2 but from experiments in which range of F_n was large, as in curves *B* and *C* of that figure. The constants a and b are from *A* to *G*: 1·25+1·0; 0·97+2·4; 1·08+1·0; 0·84+8·9; 1·17–0·6; 0·92+3·7; 0·84+8·8. Curve *H* is the average from 33 experiments; $a = 1{\cdot}043$, $b = 2{\cdot}6$ imp/sec.

abruptly together at the threshold and the former has to be raised a little before balanced states can be obtained, as exemplified in Fig. 2*H*, especially when $a < 1$. This means a negative slope in the graph of Fig. 3 and hence recurrent inhibition is largest at the low frequencies of discharge. At the upper end recurrent inhibition may approach saturation before F_n has reached its maximum value and so the slope will be changed because now $F_n = F_i$ and recurrent inhibition is gone. It was never supplanted by recurrent excitation. With some spikes the curves for F_i against

F_n (equation (1)) have flattened out at the upper limiting values. Repetition of long-lasting afferent stimulation at the maximum stimulus strengths is likely to undermine reliability (see below).

In Fig. 5 the results with 33 fibres are summarized. The peak of the minimum values for F_n is between 6 and 10 imp/sec. F_n min. should, of

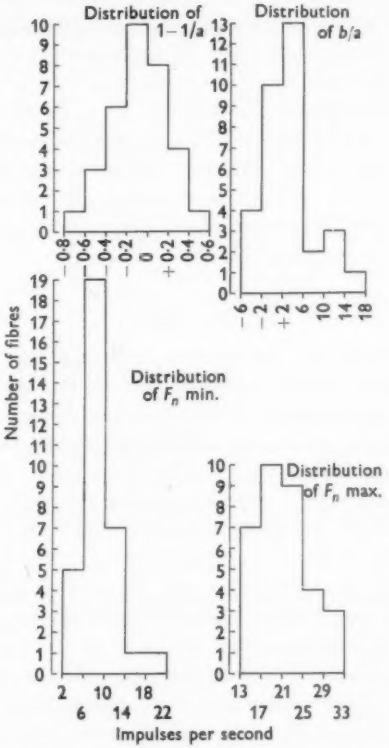

Fig. 5. Histograms showing distributions, for 33 fibres, of the various quantities indicated: class intervals correspond to the intervals marked on abscissae.

course, be taken to refer to the threshold for a maintained discharge and not to the phasic burst by which many fibres respond to very weak stimuli. The maximum is spread over a considerable range with a broad peak between 13 and 25 imp/sec. The average range ($F_n$max. $-$ $F_n$min.) is 12 imp/sec. The other two distribution curves show $(a-1)/a$ and b/a. In the present material a averaged out at about unity (1·04), so that the average difference between F_n and F_1 would be largely determined by the average value of b, which was 2·6 imp/sec. The average curve based on

these values is plotted in Fig. 4 as H. While this may serve as a descriptive landmark for the present data—a selection based on spikes that actually could be made to vary in F_n—it cannot at the moment be used as representative mean (cf. below). The really significant finding is not the average curve but the facts that (i) the rate of discharge under recurrent inhibition, F_i, tends to be proportional to the normal or control rate of discharge F_n when forced to act at that rate; (ii) the slopes in the plot of $F_n - F_i$ against F_n (Fig. 3) vary as much as they do and (iii) some of the curves for $F_n - F_i$ as a function of F_n even slope downwards (when $a < 1$ or $(a-1)/a$ is negative) so as to approach a minimum $F_n = F_i$ which corresponds to $b/(1-a)$, as seen from equation (1). The variations in slope of $F_n - F_i$ represent gradations of recurrent inhibition which we do not yet fully understand. However, being reasonably well defined by the use of a simple formula, they are easily reproducible under various conditions and so, in the end, must become explicable.

Time factors

Several experiments were devoted to investigating the possible significance of the moment of onset of recurrent inhibition relative to time of onset of afferent stimulation. Durations of afferent stimulation up to 300 sec were used, the durations of afferent stimulation in the standard experiments having been kept within 30–60 sec. During maintained iterative stimulation of the afferent nerves the spike frequency slowly declined, even though temporarily raised a little by the rebound after each inserted period of antidromic stimulation. Post-tetanic potentiation will increase during the first 10 sec (Lloyd, 1949; Curtis & Eccles, 1960) and a steady state would therefore have to be tested after a minimum of 10 sec of afferent stimulation. This also was our minimum time and most tests actually fell between 20 and 30 sec after onset of afferent tetanization. At stimulus rate 114/sec potentiation of individual cells would be modest (Curtis & Eccles, 1960). In many, perhaps in most, cases the temporal variation of the value of F_i was a simple consequence of the change of F_n and when this was so all observations fitted on to the same straight line for F_i against F_n in our plot of equation (1). With longer durations of stimulation a real change commonly took place in the sense that recurrent inhibition increased in potency. This is illustrated with a characteristic example in Fig. 6, fully explained in its legend. After some 30 sec of afferent stimulation the genuine increase of recurrent inhibition would become measurable, when present. The opposite effect that recurrent inhibition was stronger early than late in afferent stimulation was rarely encountered.

Supraspinal control

This is too large a subject to be broached in the present work but, on the other hand, it is too important for an interpretation to be wholly left out. The method of approach elaborated in the previous sections is useful in studying supraspinal control because a check of the whole curve in the plot of Fig. 2 before and during supraspinal stimulation provides a more reliable criterion than individual values can give.

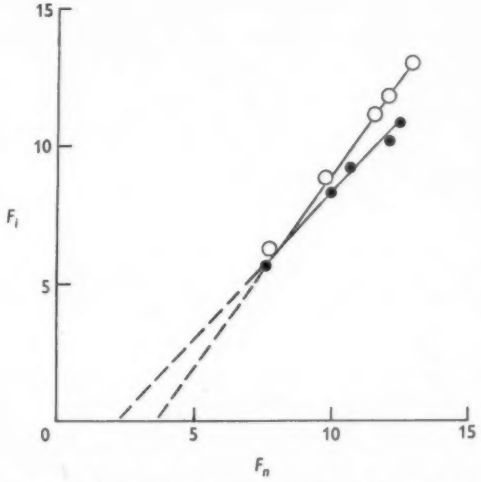

Fig. 6. Plot as in Fig. 2 for two periods of locking antidromic shock to spike at moments 30 sec (○) and 60 sec (●) after start of afferent stimulation. Co-ordinates scaled in impulses/sec.

Thus in Fig. 7, after some hours of experimentation, a spike was located which was but feebly influenced by recurrent inhibition, as is shown by curve *B* in comparison with curve *A*, which is the 45° line from zero for absence of effect or $F_n = F_1$. This spike could be made to respond by increased inhibition to antidromic stimulation when the brain was stimulated from Horsley-Clarke co-ordinates P 6, H 2, 1 mm contralateral to the mid line, as well as from the pyramid at the bottom. The order in which the stimulation periods followed is marked in the figure and it is seen that the effect was progressive from *C* to *D*. The underlined points were taken without simultaneous central stimulation, but apparently the change was slow enough to persist in the absence of such stimulation, which in these types of experiment was inserted together with brief periods of antidromic stimulation while afferent tetanization was maintained.

The main difficulty in evaluating findings of the kind shown in Fig. 7 is clearly that the change may depend upon loss of surplus excitation, but when the readings fell so well on a line as did the early ones, marked *C*, this error does not appear likely. However, no such criticism can be directed against the opposite suppressive effect of supraspinal stimulation, illustrated in Fig. 8. The open circles refer to averaged readings taken *before* and *after* a period of stimulation of the cerebellar point shown in the figure,

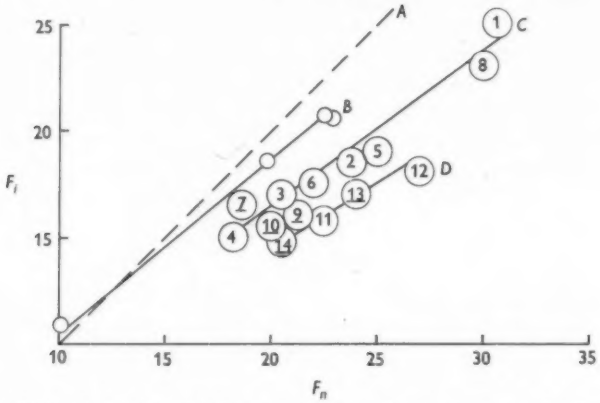

Fig. 7. Plot as in Fig. 2. All data from one fibre. *A*: to show graph for $F_n = F_i$. *B*: initial control for fibre drawn to fit $F_n = 1{\cdot}22\ F_i - 2{\cdot}8$. *C* and *D* successive periods of lower frontal portion of anterior lobe of cerebellum at Horsley-Clarke co-ordinates P 6, H 2 just contralateral to mid line at frequency 300/sec and 9·0 V; coaxial electrode, insulated tip referred to its shield. Order of observations marked on graph; underlined numerals are observations without stimulation of cerebellum. Curve *C* drawn to $F_n = 1{\cdot}39\ F_i - 2{\cdot}8$, *D* to $F_n = 1{\cdot}58\ F_i - 2{\cdot}8$.

the filled circles to readings taken during such stimulation. The difference in slope is not significant but the constant b is shifted from 8·2 to 4·0 imp/sec during stimulation of the inhibitory region. With a slope of the order of 1·0 this means that at each frequency the effect of recurrent inhibition, $F_n - F_i$, has been diminished by roughly 4 imp/sec owing to co-stimulation of the brain stem.

It is therefore clear that by our index there are neural or (possibly) hormonal governors of the Renshaw cells and so averaging of our data can only be of interest for the sake of surveying them conveniently and not of disclosing any meaning in them that would be lost among individual variations. At the moment we are engaged in studying supraspinal effects by recording from Renshaw cells. Koizumi, Ushiyama & Brooks (1959), using this approach, found spontaneously active cells, which they held to be Renshaw cells, to be inhibited by stimulation of the reticular formation.

More experience by this and the present method is needed to ascertain whether or not Renshaw cells and thus recurrent inhibition are controlled merely indirectly by variations in type, number or discharge rate of their motoneurones, or directly as are so many other interneuncial cells. Our results suggest the latter alternative. Holmqvist & Lundberg (1959) did not find any evidence of supraspinal control of Renshaw cells by monosynaptic testing but their curves do differ for spinal and decerebrate cats.

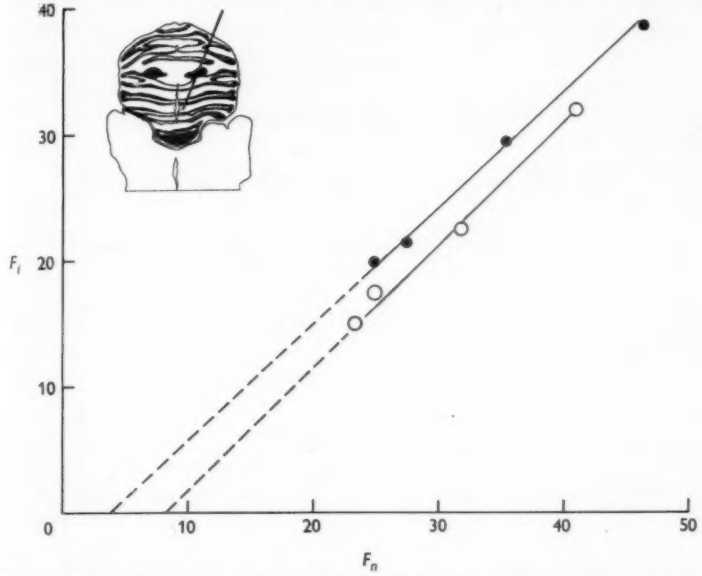

Fig. 8. Afferent stimulation maintained for 50 sec during which time antidromic shock was locked to discharging spike for brief intervals only, as tests. Values from such tests were averaged over four consecutive 10 sec periods from control runs (○) before and after one series during stimulation (●) of the point in the frontal part of the anterior cerebellum illustrated as inset. This well isolated spike had an exceptionally high initial frequency of discharge of around 40/sec, falling during maintained afferent stimulation to values between 20 and 25 imp/sec. The point stimulated proved to be in the anterior lobe of the cerebellum (electrocoagulated) as shown by inset where it is marked by pointer. Stimulus frequency during experiment was 300/sec, strength 0·45 V through tip of thin coated needle against ground. On increasing stimulus strength the discharging spike itself was ultimately inhibited. The difference between constants a is not significant (1·07 and 1·03) but the constant b is 8·2 in the control (○) and only 4·0 (●) during stimulation. Co-ordinates scaled in impulses/sec.

Frequency limitation

We recall from the previous paper (Granit & Rutledge, 1960) that F_n is a function of the depolarizing pressure, defined as $(P_{dep} + P_{pol})$, the latter

factor including all 'natural' inhibitions (ortho- as well as antidromic ones) and also after-hyperpolarization. It would be easier to measure F_n as a function of depolarizing pressure if these inhibitions, undesirable here, were absent or could be kept constant. Barron & Matthews (1938) tried to avoid them by stimulating motoneurones from outside. The only figures they publish (one motoneurone) suggest that F_n is directly proportional to net depolarizing current (= depol. pressure). Pascoe (1957) and M. G. F. Fuortes and K. Frank (personal communication) have stimulated cat motoneurones from the inside, using the bridge technique of Araki & Otani (1955). They confirmed the old findings, Fuortes & Frank in particular

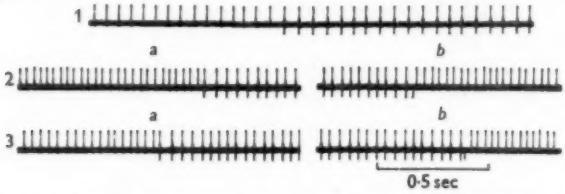

Fig. 9. Records showing maximum frequency obtainable by afferent stimulation of gastrocnemius nerves (1) and same when in addition fore part of body was squeezed, twice repeated (2, 3). Antidromic stimulation locked to discharging spike in all cases between *a* and *b* as controls.

stating explicitly that 'five normal-appearing motor horn cells gave the following slopes: 4, 5, 6·2 and 13·6 imp/sec/mμA' and that many cells went up to rates of 100 imp/sec along straight lines. Thus, if depolarizing pressure is allowed to rise, F_n rises in strict proportion, how far depending upon the cell and thus upon a factor of selection.

With stimulation across synapses there is an unknown amount of P_{pol} from inhibition and therefore we have to write

$$F_n = \text{const} \times (P_{dep} + P_{pol}). \qquad (3)$$

From this follows directly that there are two fundamentally different possibilities for limitation of frequency of discharge: (i) F_n itself may be limited and the cell may therefore fail to respond to progressively increasing depolarizing pressures, or (ii) the depolarizing pressure may be the regulated quantity. For both alternatives we are free to hypothesize mechanisms (see Discussion).

Our results uniformly show that the controlled quantity is the depolarizing pressure but they cannot wholly exclude alternative (i). Thus the experiment of Fig. 9 is a simple but instructive case presenting a motoneurone whose F_n could not be raised beyond 20–22 imp/sec by stimulation of muscular afferents (record 1) while to stimulation by manipulation of forelimbs and their skin it fired (in 2 and 3) at rates of

30–34 imp/sec. Clearly, therefore, the depolarizing pressure was limited for the muscular afferents only (representing one particular combination of excitation and inhibition). The discharge mechanism itself was perfectly capable of raising F_n in response to a rise of depolarizing pressure.

As all experiments in this work were run from $F_n$min. to $F_n$max., they can all be reconsidered here from the point of view of frequency limitation. Since recurrent inhibition is a controlled event it may well be governed so as to become the limiting factor in many cases. This is a different proposition, however: in the present work, we have to keep within the precincts of our own measurements and from them some general conclusions emerge which are of interest for the general problem of stabilization.

Thus, when $a = 1$ and recurrent inhibition is independent of F_n, being equal to b, it is immediately clear that recurrent inhibition cannot have been the decisive limiting influence. Within the range from $F_n$min. to $F_n$max. depolarizing pressure has risen and the effect of the rise is stopped by the sum total of restricting factors (see p. 314).

When $a > 1$ the effect of recurrent inhibition increases with F_n, i.e. depolarizing pressure, but there is nothing to suggest that this process could not have gone on beyond the actual maximum F_n obtained, had it been possible to increase depolarizing pressure from the muscular afferents. In both these cases natural recurrent inhibition may be assumed to have added its contribution to frequency limitation, but it has not by itself had a decisive influence on determining $F_n$max.

However, when a is a good deal below unity, the slope of the curves for $F_n - F_i$ against F_n is negative and this means that recurrent inhibition is at its best at low rates of firing of the motoneurone. It thereafter approaches a limit $F_n = F_i$, as F_n increases. The limit is defined from equation 1 as $b/(1-a)$. In such cases, if this value agrees with the maximum F_n obtained for the same motoneurone, recurrent inhibition may well have been the decisive limiting factor. On this interpretation F_n approached F_i because 'natural' and experimental recurrent inhibition together set a limit for F_n. In this case F_n max must not exceed the calculated limit $F_n = F_i$, given by $b/(1-a)$. Results comparing calculated and observed values of F_n max. for 8 fibres are tabulated in Table 1.

Table 1 shows that the upper limit of F_n may well have been determined in these cases by recurrent inhibition. Incidentally it throws doubt on the view that the recurrent excitation of Wilson (1959) and Wilson, Talbot & Diecke (1959, 1960) is much of a complication in our type of experiment, as there is no obvious reason why in its presence F_n should be limited to the low values obtained and, moreover, to the very values required by recurrent inhibition as a major limiting factor. One would expect to observe the recurrent excitation occasionally in the experiments, if it were

a really important mechanism in extensors, in particular in the region of F_n max. As a matter of fact one either finds inhibition with rebound or no effect whatsoever. The relation to rebound was dealt with in the preceding paper (Granit & Rutledge, 1960).

Stabilization of rate of firing should also be considered from the point of view of how motoneurone discharges are limited downwards. This at least is a situation that should give ample play for recurrent inhibition, if for no other reason because excitatory drive is reduced and with it the resistance of the motoneurone to the inhibitory barrage from the Renshaw cells (Granit & Rutledge, 1960). In fact, when firing at slow rates is a desideratum, as in the slow soleus, it can only be obtained if heavy afferent projections antagonize recurrent inhibition or else, at these slow rates, there would not be enough surplus excitation to counteract recurrent inhibition.

TABLE 1

Frequency range	$(a-1)/a$	F_n max. Observed	F_n max. Calculated
7	−0·43	13	12
9	−0·22	17	19
10	−0·41	18	20
10	−0·50	29	35
11	−0·22	21	24
7	−0·57	13	13
4*	−0·23	10	11

* Not included in summary of Fig. 5 on account of the small frequency range.

In the present experiments cells were commonly encountered among the triceps motoneurones which it proved possible to drive at slower rates with recurrent inhibition than without it. No doubt rebound also aids in stabilizing of the rhythm at these low frequencies. Now Granit, Pascoe *et al.* (1957) found that the small tonic α motoneurones of Granit, Henatsch & Steg (1956) were especially well provided with recurrent inhibition. The cells wholly lacking it tended to be found among the phasic motoneurones, though many of these also possess this mechanism. Since the γ spindle control is so strong on the small tonic motoneurones, Granit, Pascoe *et al.* (1957) drew the same conclusion as we now have arrived at from other points of view, namely that slow steady rates of discharge require good drive and strong recurrent inhibition. Eccles, Eccles & Lundberg (1957) next proved by the intracellular method that the soleus motoneurones, as the typical representatives of the small α cells, had excitatory projections from the large spindle afferents some 25% stronger than had the phasic alphas. Finally Kuno (1959) and R. M. Eccles, A. Iggo and M. Ito (personal communication) have confirmed by testing different types of motoneurones that recurrent inhibition is particularly strong on soleus motoneurones compared, for example, with gastrocnemius neurones.

This series of interconnected experiments and deductions give recurrent inhibition a definite role as stabilizer of the slow rhythm of the stretch-sensitive tonic α motoneurones and thereby also serve to characterize the latter functionally defined system by properties other than size of efferent fibres (Granit *et al.* 1956, Eccles *et al.* 1957, 1958; Granit, Phillips *et al.* 1957; Henneman, 1957), sensitivity to post-tetanic potentiation (Granit *et al.* 1956) and after-hyperpolarizations of long duration (Eccles *et al.* 1958) It is known that soleus motoneurones can be stabilized at rates as low as 5 or 6/sec (Denny-Brown, 1929; Granit, 1958, fig. 1). To prevent misunderstanding it should be noted that by electrical stimulation, as in this work, or by activating the muscle spindles with succinylcholine (Granit, Skoglund & Thesleff, 1953) α motoneurones can be made to discharge tonically which normally do not do so (Henatsch & Schulte, 1958).

In several animals we have encountered the combination of heavy stabilization with failure of recurrent inhibition. Some but not all of them might be called unsuccessful preparations. Considering that the Renshaw cells are controlled, excessive stabilization may be one aspect of the activity of such governors and so, practically speaking, discharge frequency might become an invariant. On the other hand, as we shall see (cf. Discussion), many factors may contribute to stabilization, and with regard to recurrent inhibition itself it is not always possible to decide in individual cases whether it fails to appear because the mechanism is over-engaged or because it is feeble. At the moment we do not know why in some preparations excessive stabilization occurs, in the sense that the range F_n max. $- F_n$ min. is compressed to a few impulses per second.

DISCUSSION

The main finding of this work is that recurrent inhibition is proportional to discharge frequency, which in its turn is proportional to depolarizing pressure (cf. p. 322). This being so, the efficacy of recurrent inhibition, defined as $F_n - F_1$, will depend not only upon the number of overlapping Golgi recurrent collaterals and Renshaw cells taking part in regulating the discharge of any one motoneurone but also upon the extent to which one motoneurone differs from another in respect of the constant of equation 3. Fuortes & Frank (cf. p. 320) found this constant to vary from 4 to 13·6 imp/sec/mμA. To understand what this means, assume that we have isolated two motoneurones both of which discharge to afferent stimulation at identical rates, F_n. Assume further that in the two cases recurrent inhibition as such produces exactly identical repolarizing currents P'_{pol}. In terms of discharge frequencies the effects of recurrent inhibition (F_1) will then have to be in the ratio of 4 to 13·6 for the two motoneurones. By the

present technique we cannot measure this factor, but only suspect that it is of major importance inasmuch as small constants are likely to be found in tonic slowly-discharging cells and large ones in cells that are firing rapidly. However, fundamentally this prediction is verifiable by experimentation.

At the moment it is well to realize that terms such as 'strong' or 'weak' recurrent inhibition can only apply to one and the same motoneurone in different states, since no absolute measure is available. Clarification of this point, however, is merely a matter of further experimentation.

An interesting parallel may be drawn with the results of Hartline & Ratliff (1956) obtained with Hartline's (1949) lateral inhibition in the eye of the horseshoe crab, *Limulus*. This process was found to be proportional to discharge frequency, implying that if one ommatidium A is steadily illuminated to deliver a constant rate of firing (F_n) from its eccentric cell, the illumination of a nearby ommatidium B will inhibit that discharge to F_i in strict proportion to the rate F_x at which B is made to discharge. There are no internuncial cells in *Limulus*, the contacts are held to be axo-axonic (H. W. Miller, personal communication), and the individual factors can be better isolated than in our case where the effect is competing with 'natural' recurrent inhibition for the output from the Renshaw cells. Nevertheless, the fact that the linear frequency rule is so well obeyed in our case also must reflect some basic similarity. It is clear that the degree to which the Renshaw cells are controlled from above and the circumstances in which they are switched on and off are important data, and that without this information it would be unwise to carry interpretation too far. At the moment the greatest difficulty in the way of a uniform explanation of the results of measuring $F_n - F_i$ as a function of F_n is that some curves have negative slopes. We would at the moment interpret such variations of slope by assuming different degrees of competition on the part of natural recurrent inhibition rather than by ascribing them to complications from recurrent excitation, as discussed in the previous section.

With regard to 'stabilization' or frequency limitation, either term is little more than a collective name for the integrated effect of several processes engaged in the regulation of the depolarizing pressure. Without further discussion of details we would like to mention (*A*), anatomical limitation of afferent terminals, (*B*), afferent inhibition, (*C*) recurrent inhibition, considered above in the Section of stabilization and hardly deserving a lengthier discussion before further data on regulation and control of the Renshaw cells have become available. (*D*) Desensitizing to repetitive stimulation by the postulated transmitter (see e.g. Thesleff, 1959) should be considered but does not seem important in our case with cells firing for many minutes at slow rates, and should more likely be considered with

alternative (i) of the preceding section (see also below). (*E*) After-hyperpolarization was noted by Brock, Coombs & Eccles (1953) and especially discussed by Eccles *et al.* (1958). The idea that after-hyperpolarization sets the upper limit of frequency of discharge finds its strongest support in the fact disclosed by the latter authors, namely that the slowly firing soleus motoneurones have especially long after-potentials. On the other hand, it is perfectly clear that after-hyperpolarizations can be overcome by sufficient depolarizing pressure, or else the results with direct electrical stimulation of motoneurones could never have been obtained. Also, large α motoneurones and small γ motoneurones (J. C. Eccles, R. M. Eccles, A. Iggo and A. Lundberg, personal communication) have after-potentials of the same order (70 msec). Yet the latter can fire at 10 times faster rates than the former. Finally, after-hyperpolarization is generally measured within the soma, whereas the firing region equally generally is held to be the area between axon hillock and medullated portion of the motoneurone (Araki & Otani, 1955; Coombs, Curtis & Eccles, 1957*a*, *b*; Fuortes, Frank & Becker, 1957). After-hyperpolarization is considerably shorter for the spike that arises in this segment (Brock *et al.* 1953). We are convinced that after-hyperpolarization alongside with other factors decisively contributes to frequency limitation (upwards), but would think factors *B* and *C* (above) more important. However, the slowly firing soleus motoneurones must make use of the added effects of recurrent inhibition and after-hyperpolarization suggesting that for extreme degrees of stabilization this combination, as again R. M. Eccles, A. Iggo and M. Ito (personal communication) have found, is likely to be of dominating importance. Perhaps, indeed, recurrent inhibition stabilizes only inasmuch as it co-operates with after-hyperpolarization.

While the factors hitherto enumerated belong to the governors of depolarizing pressure, the alternative ((i) in preceding Section), that F_n itself could be cut in range in spite of increasing depolarizing pressure, might be realized by accommodation. Pascoe (1957) and Frank & Fuortes (1960) in their experiments on stimulation of motoneurones from the inside found the discharge to undergo some accommodation leading to lower rates of firing during maintained stimulation. We have seen the same effect regularly in the present work, though in our case the slow decrease of discharge frequency could also be otherwise explained. Now Araki & Otani (1959) have reported that, with intracellular electrodes in the motoneurones of toads, the firing region around the axon hillock accommodates considerably faster than the soma. If this were so in the cat also, the consequence would be that more depolarizing pressure would be needed to maintain any given frequency of discharge. Thus, in this case, F_n would be cut and not depolarizing pressure. In

terms of our measurements this would mean that recurrent inhibition, removing a constant fraction P'_{pol} of the depolarizing pressure, would have a stronger effect later in the discharge than in the beginning. This finding was actually fairly regular and it was illustrated in Fig. 8. In many neurones this effect of stimulus duration would be late in appearance or absent but in some it could be seen within the first 30 sec of afferent stimulation.

'Stabilization' as a biological concept in relation to discharge rate of motoneurones may seem complex, but it is in the nature of this concept to be tied up with the influences that occur as components of the general problem of regulation in its widest sense and so it must always reflect their integrated complexity. It is perhaps better supplanted as a term by 'frequency-limitation', as this would avoid unjustified electronic analogies. Can such a complex concept serve as a useful entity deserving experimentation on its own account? The best case that can be made out for a positive answer is the existence of strong recurrent control and long-lasting after-hyperpolarizations in the small tonic α neurones, which for theoretical reasons were assumed to be highly stabilized and actually proved to be so by experimentation, as discussed in this and the previous Section.

SUMMARY

1. Tetanization of gastrocnemius afferents at rate 114/sec at varying strengths was used to set up a tonic reflex discharge from extensor motoneurones, isolated in root filaments. Selecting motoneurones that were capable of varying in discharge rate, the firing spike was made to trigger an antidromic shock to the rest of the root, and thus recurrent inhibition could be studied as a function of frequency of firing.

2. The discharge rate under recurrent inhibition was found to be related linearly to the average rates of discharge in the controls just before and after antidromic stimulation.

3. The constants of the linear curves were determined for 33 motoneurones varying in range of frequency by more than 6 imp/sec.

4. These constants could be modified by supraspinal stimuli.

5. The effect of recurrent inhibition tended to increase as a function of duration of maintained afferent stimulation.

6. Maximum and minimum maintained rates of discharge were determined for the 33 motoneurones and the factors responsible for 'frequency limitation' discussed on the basis of the data obtained.

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THE METABOLIC RATE AND HEAT LOSS OF FAT AND THIN MEN IN HEAT BALANCE IN COLD AND WARM WATER

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Shivering can be produced by the return of cold blood from the limbs (Sherrington, 1923–4; Uprus, Gaylor & Carmichael, 1935; Glaser & Holmes-Jones, 1951) but observations that it can be produced by surface cooling alone (Jung, Doupe & Carmichael, 1937; Davis & Mayer, 1955; Good & Sellers, 1957; Spurr, Hutt & Horvath, 1957) suggested that deep cooling might be of little importance in practice. However, it has been reported (Keatinge, 1960*a*) that although the rate at which men's rectal temperatures fell in water at 15° C was closely related to their subcutaneous-fat thickness, their early metabolic response to the cold immersion was not, and the metabolic rates of thin men only rose substantially above those of the fat men during the later part of immersions, when the thin men's rectal temperatures fell. The present studies of fat and thin men in a steady state of heat exchange in water were designed partly to confirm or refute this evidence that stimulation of deep temperature receptors plays a major part in adjusting the metabolic rates of fat and thin men to their different rates of heat loss during prolonged exposures to cold. They were also designed to show whether fat and thin men have different critical ambient temperatures, at which physical temperature regulation is complete and below which the metabolic rate is increased (Rubner, 1902; Burton & Bazett, 1936; Scholander, Hock, Walters, Johnson & Irving, 1950).

It was hoped that these experiments would also show whether cold vasodilatation substantially reduced the tissue insulation of fat men in near-freezing water. This seemed probable, as cold vasodilatation takes place eventually in cold extremities of even generally chilled people (Keatinge, 1957) and appears to be due largely to the direct effect of low temperatures on blood vessels (Keatinge, 1958). Physical exertion accelerates the fall in the rectal temperature of thin men in water at 16° C (Pugh & Edholm, 1955) and in water at 5 and 15° but not at 25 or 35° C (Keatinge, 1959) and shivering may cause a fall in the total body insulation (Burton & Bazett, 1936; Carlson, Hsieh, Fullington & Elsner, 1958). It was hoped that the present experiments would provide information about

the effect of shivering on the tissue insulation of fat and thin men, and additional experiments were made to determine whether work assisted the maintenance of the body temperature of either fat or thin men in water just too cold for them to maintain thermal stability when still, since this question is of practical importance.

A preliminary communication (Keatinge, 1960*b*) of some of these and of some earlier results has been made already.

METHODS

The subjects were eight healthy naval ratings aged between 17 and 21, none of whom had recently been exposed to an unusual degree of cold. They were volunteers and were selected to include both fat and thin men. On arrival each was given a medical examination, his height and weight was determined, and his skin-fold thicknesses measured with Harpenden callipers at four sites (Keatinge, 1959). Each man was repeatedly immersed over a period of one week in water at temperatures ranging from 38° C down. The food intake was not restricted before immersions. The immersions at 38, 36 and 34° C, which were made first, were 'crossed over' as far as possible, but those at lower temperatures were not. Six of the eight men were immersed in water at 38° C until their temperatures had become stabilized, but the other two subjects, 3 and 7, became so uncomfortable that they had to be allowed out before this, so that no values for insulation of these two men at this temperature could be calculated. Subjects 2 and 3 were unwilling to go into water colder than 28° C but the six others were all immersed at progressively lower temperatures, until a water temperature was found at which their temperatures were still falling steadily after 2½ hr in the water, or at an accelerating rate at the end of somewhat shorter immersions. Five of these men were immersed again at this last temperature and were told to work as hard as possible on this occasion. The experiments were made during October, November and December.

The immersions were performed in an indoor tank, 8 × 4 × 4 ft. (3·6 m³). The subject sat in the tank on a slatted wooden seat wearing only a pair of very brief bathing trunks and a large fleece-lined helmet which covered much of the face and the whole of the rest of the head. The water just covered his shoulders and was vigorously stirred by a mechanical device; the temperature of the immersed skin has been shown to be within about 1° C of water temperature under these conditions, even in water as cold as 5° C, within 20 min after immersion (Keatinge, 1959). The water was maintained within 0·1° C of the required temperature by electrical heating or refrigeration, and the room temperature was maintained by electrical heating at 25 ± 1° C. In the few 'working' experiments the work consisted of a rowing movement, with the subject on a sliding seat in the water and with his feet attached to the end wall of the tank. This tank and accessory apparatus has been described in detail (Keatinge, 1959).

Rectal temperature was measured by a thermojunction enclosed in semi-rigid plastic tubing inserted 11 cm from the anus. The cold junction was in a vacuum flask of ice and water, and the e.m.f. was measured by a potentiometer. Readings were accurate to the nearest 0·05° C. Stability of the rectal temperature was defined arbitrarily as the absence of a change greater than 0·1° C during any period of 30 min starting at least 30 min after the man was immersed. A change of 0·05° C or less in 15 min was, however, accepted in some immersions at the lowest temperatures, if the subject was very anxious to leave the water. The collection of expired air for determination of the metabolic rate was restarted repeatedly until the rectal temperature was found to have been 'stable' throughout the period of collection.

During metabolic-rate determinations the subject was fitted with a mouth-piece and nose-clip. The men wore these for 10–20 min before the first immersion, to get used to them. The

inspired air was led to the subject from outside the building through a copper duct which warmed it to room temperature. The expired air was led from the subject through a Max-Planck Institute Respirometer which measured its volume, and the oxygen deficit of the gas was analysed by an automatic analyser (Hartmann und Braun, Frankfurt-am-Main). The metabolic rate was then calculated by the method of Weir (1949) from the volume of expired air corrected to s.t.p. and its oxygen deficit.

Tissue insulation is expressed as the reciprocal of the kilocalories dissipated per square meter of immersed skin per degree centigrade temperature gradient between the rectum and the water. This was generally calculated when the men were in a steady state of heat exchange, assuming the rate of heat loss to be the metabolic rate minus the heat loss due to evaporation and warming of the inspired air from room temperature to body temperature. This air was drawn from outside the room. The outside day temperature was always between 0 and 10° C and the humidity 85–100 % during the period of the experiments. The air was found to be warmed to 25 ± 1° C when inspired. In calculating the respiratory heat loss, the air was assumed to be 90 % saturated with water vapour at 5° C when drawn from outside, to be at 25° C when inspired by the subject and to be almost saturated with water vapour at 37° C when expired (Christie & Loumis, 1932). The errors introduced into the calculated body insulation by making these assumptions are unlikely to have exceeded ± 2 %. The total surface area of the men was obtained from their height and weight by the nomogram of Hawk, Oser & Summerson (1947). Since the head represents approximately 6·6 % of the surface area of men (Sawyer, Stone & Dubois, 1916) the area of the men's skin immersed in the water was taken as 93·4 % of the total skin area. Heat loss from the head, which was covered by a fleece-lined helmet, was assumed to be negligible. In a few experiments at low temperatures, in which the men's temperatures failed to become stabilized, approximate values for tissue insulation were calculated. In calculating these allowance was made for the loss of stored heat from the deep tissues of the body, assuming these to represent 64 % of the total mass of the body and to have a specific heat of 0·83 (Burton, 1935), and their fall in temperature to be that measured in the rectum.

In many experiments heat-flow disks (Hatfield, 1949) were stuck by 'Nobecutane' (Evans) to the dorsum of the terminal phalanx of the right index finger, the middle of the flexor surface of the forearm, the skin over the sternum at the level of the 4th intercostal space, the abdomen just above the umbilicus, and the dorsum of the right foot. Since difficulty was experienced in making the disks stick securely for long periods they were supported by loose rubber bands round their bases in the first two positions, and in the others were held on by the tips of wooden pointers while readings were taken.

RESULTS

Table 1 gives the skin-fold thickness of the subjects together with their heights and weights, total surface areas and the surface areas immersed during the experiments. The subjects are numbered in the order of their skin-fold thicknesses. The first four had similar mean skin-fold thicknesses, between 6·5 and 8·5 mm, while subjects 7 and 8 who appeared obese had mean skin-fold thicknesses of 26·7 and 26·8 mm.

Figure 1 shows the metabolic rate of each subject when his body temperature had become stabilized in water at different temperatures. The metabolic rate of every man began to increase when the temperature of the water was lowered below about 33° C, but the rise was much steeper with the thin than with the fat men, whose metabolic rates rose steeply only in

water below 12° C. The figure also shows that the men's metabolic rates rose when the water temperature was increased above 34° C.

TABLE 1. The heights, weights, skin-fold thicknesses, surface areas and immersed surface areas of the men (Subjects numbered in order of their mean skin-fold thicknesses.)

Subject	Height (cm)	Weight (kg)	Skin-fold thickness (mm) Biceps	Sub-scapular	Abdomen	Sub-costal	Mean	Total surface area (m^2)	Surface area immersed (m^2)
1	173	69	4·1	7·1	7·8	7·2	6·5	1·82	1·70
2	162·5	54·5	3·1	8·2	8·6	7·0	6·7	1·58	1·48
3	171	64·5	3·5	9·8	7·8	6·8	7·0	1·76	1·64
4	187	79	3·4	10·2	11·9	8·4	8·5	2·04	1·91
5	170·5	75	4·4	14·0	13·4	13·15	11·2	1·87	1·75
6	168	69	4·9	13·5	18·9	15·1	13·1	1·78	1·66
7	175	89	9·3	35·4	31·1	30·9	26·7	2·05	1·92
8	170	89	10·5	35·3	28·7	32·9	26·8	2·00	1·87

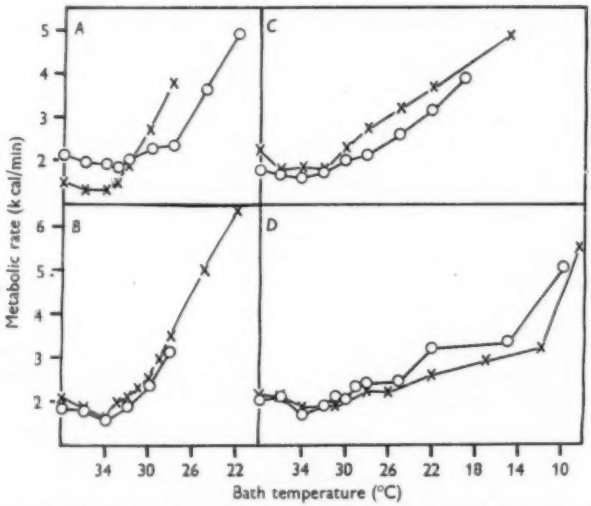

Fig. 1. The effect of the bath temperature on the metabolic rate of fat and thin men in a steady state of heat exchange. Subjects numbered according to fat thickness (higher numbers = fatter men)

Block...	*A*	*B*	*C*	*D*
Subjects ○–○	1	3	5	7
Subjects ×–×	2	4	6	8

Figure 2 shows the total body insulation of each subject at various water temperatures. It was at a minimum at the highest temperatures, and this minimum insulation was about the same for all the men, being as low in fat as in thin men. As the water temperature was lowered the insulation rose to a maximal value which was much higher in fat men than in thin

men, and was reached at a lower water temperature with fat than thin men. With a further fall in water temperature the insulation fell. This decrease in insulation from the maximal values was of about the same size in all except the two fattest men, whose insulation fell sharply in water below

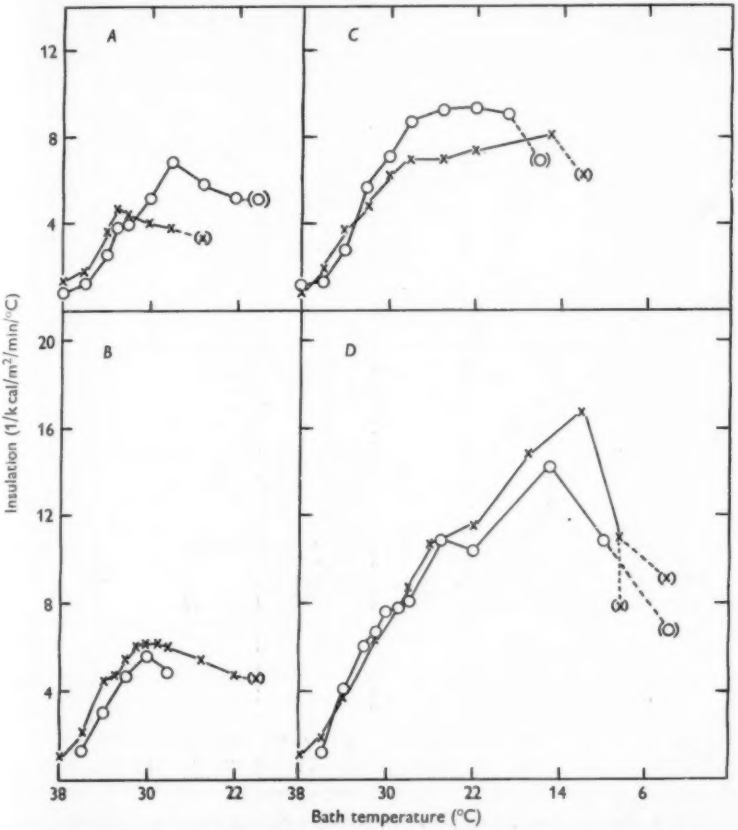

Fig. 2. Total body insulation of fat and thin men in a steady state of heat exchange in relation to bath temperature. Symbols and blocks as in Fig. 1. Brackets indicate approximate values when temperature failed to become stabilized.

12° C. Two values are given for subject 8 in water at 8° C; the higher was obtained when his temperature was stabilized temporarily after 75–90 min in the water, and the lower was an approximate figure calculated after his temperature had begun to fall again.

Figure 3 shows that there was an approximately linear relationship between bath temperature and stable body temperature in all except the

fattest men. In the thinner men the slope of the line was generally significantly different from zero when the t test was applied (subject 4, $P < 0{\cdot}005$; subjects, 1, 5 and 6 $P < 0{\cdot}05$). The slope of the line was less steep for the moderately fat than for the thin men, and the two fattest men

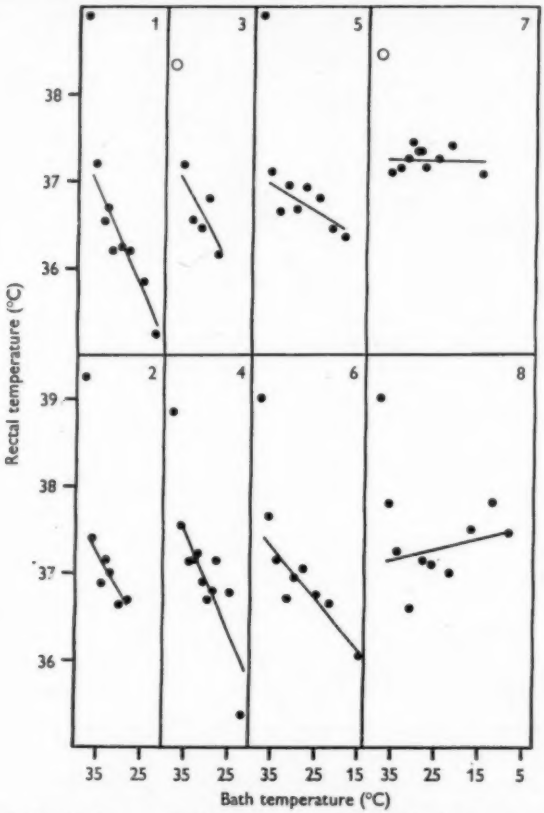

Fig. 3. Relationship between bath temperature and stable body temperature in fat and thin men. Number in each section indicates the subject, higher numbers = fatter men. (○) shows where the experiment had to be terminated before the rectal temperature had become fully stabilized.

showed no tendency to a lower body temperature as the bath temperature was lowered; the temperature of one of them even tended to be higher at the lower bath temperatures. The results from the 38° C immersions were excluded from these calculated lines, as this temperature was higher than the men's normal body temperature and their body temperatures were forced to rise.

Figure 4 gives the rates of heat loss measured by heat-flow disks at various sites when the men's temperatures had become stabilized in water at 38, 31–33 and 22° C. These are expressed as thermal conductivity, as heat loss related to the temperature gradient between the rectum and the bath, in order to facilitate comparisons at different water temperatures. A complete set of readings was taken at all these temperatures in only four men, two of whom were thin and two moderately fat, and the means of these are connected by a line. In water at 38° C thermal conductivity was

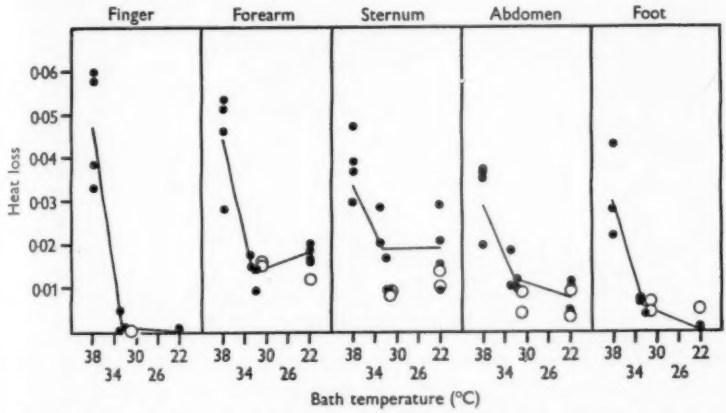

Fig. 4. Heat loss from five sites in a steady state of heat exchange in water at different temperatures. ○, two fattest men; ●, subjects 1, 4, 5 and 6 (the lines connect mean values for these four men). Heat loss shown in cal/cm²/min/° C rectal —bath temperature gradient.

high between the rectum and all parts of the body surface, and was somewhat greater to the finger than to the skin of the trunk. In water at 31–33° C conductivity to all skin areas was lower, but was much lower to the extremities than to the trunk. In water at 22° C heat loss from both the finger and foot was negligible, but thermal conductivities to the skin over the sternum were as high, and those to the skin of the abdomen almost as high, as in water at 31–33° C, while those to the forearm were generally higher at 22 than at 31–33° C. Heat loss from the two fattest men was only recorded in water at 31 and 22° C, when heat loss from their trunk tended to be less than in thin men.

Figure 5 gives the results of an experiment on a fat man (subject 8) immersed in water at 5° C for 80 min. Between 20 and 70 min after immersion his rectal temperature fell slowly but fairly steadily, his metabolic rate increased steadily, and his visible shivering also increased in intensity; this pattern of change was similar to the changes observed when

thin men were immersed in water at 20–25° C. Heat loss fell within 40–50 min to fairly low steady values from the trunk, forearm and foot, but in the finger, after falling rapidly at first, it returned in waves and 79 min after immersion reached a level higher than from any other site. When the heat loss from the finger rose to high levels the rectal temperature began to fall more rapidly. At this time the man started shivering intensely and became

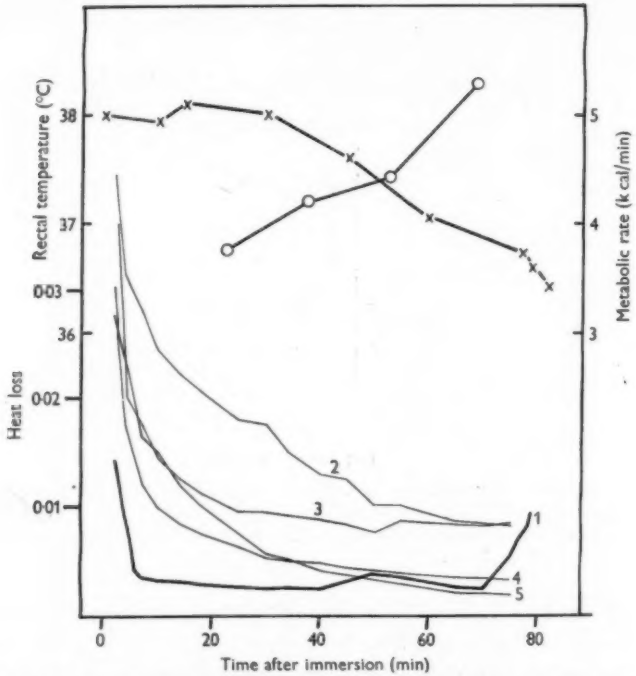

Fig. 5. Metabolic rate (○), rectal temperature (×) and regional heat loss (units as in Fig. 4) in a fat man (subject 8) in water at 5° C. Heat loss from finger 1, forearm 2, sternum 3, abdomen 4, foot 5.

very uncomfortable, and the experiment had to be terminated at 82 min. Subject 7's immersion at 5° C followed a similar course, but his finger blood flow rose earlier and the experiment had to be terminated at 40 min. When subject 8 was immersed at 8·5° C, his temperature became stabilized temporarily between 60 and 75 min after immersion, after which there was a small but distinct cyclical increase in his finger heat loss. His rectal temperature then fell again and did not become stabilized until the experiment was terminated after a total of 105 min. A similar small increase in finger heat loss was observed when subject 7 was immersed at 10° C, but

increases in finger heat loss were not observed in immersions at higher temperatures except occasionally, in association with waves of vasodilatation throughout the skin of the whole body.

Table 2 shows the effects of maximal work on the rate of fall of rectal temperature in five subjects immersed at water temperatures at which they were found to be just unable to stabilize their rectal temperature when still. In all cases their temperatures fell more rapidly when they worked than when they were still.

TABLE 2. The effect of work on the fall in rectal temperature of fat and thin men in water just too cold for them to achieve thermal stability when still

Subject	Bath temperature (° C)	Duration of periods of immersion over which comparisons were made (min)	Fall in deep body temperature (° C)	
			Still	Working
1	20	55	1·50	2·25
4	20	55	0·90	2·10
6	12	70	1·35	1·70
7	5	30	0·40	0·65
8	5	30	0·00	0·25

DISCUSSION

Part played by superficial and deep temperature receptors in the metabolic response to cold

The fact that the thin men's rectal temperatures, unlike those of the fat men, were stabilized at a lower level in cold than in warm water provides evidence that stimulation of deep receptors was largely responsible for the fact that they had higher metabolic rates than fat men in a steady state of heat exchange in cold water at a given temperature. It is known (Spealman 1946; Carlson, 1954; Keatinge, 1960*a*) that a change in deep body temperature greatly alters the metabolic response to cutaneous cold. The present experiments therefore confirm earlier evidence (Keatinge, 1960*a*) that the adjustment of the metabolic rate of individuals with differing thicknesses of subcutaneous fat to their widely differing rates of heat loss during prolonged exposure to cold is brought about largely by deep temperature receptors. Observations that the rectal and mouth temperatures of men approaching thermal stability were lower in a cold than a warm room were made by Hardy & DuBois (1938) and by Glaser & Newling (1957), but the fat thickness of their subjects was not recorded by these authors.

The metabolic rates of fat as well as of thin men in a steady state of heat exchange rose in water below about 33° C, and since the stable rectal temperature of the two fattest men was as high in cold as in luke-warm water, their relatively small increase in metabolic rate in cold water must have been a response to cutaneous cold only. This makes it clear that

cutaneous receptors are important in the metabolic response to prolonged as well as to brief exposure to cold and are capable of responding for long periods at an approximately constant low temperature. In the thin men the response to cutaneous cold was clearly reinforced by stimulation of deep receptors, and the fact that the metabolic rate of a fat man (Fig. 5) rose as his rectal temperature fell in water at 5° C shows that deep temperature receptors can be important in raising the metabolic response of fat men, too, during long exposure to really low temperatures.

The concept of 'critical temperature'

Although the fat men increased their metabolic rates in water below about 33° C, they did not achieve their maximum tissue insulation until the water temperature was much lower. Their increase in metabolic rate was therefore unnecessary, in that they could have achieved thermal balance by vasoconstriction alone at these temperatures. Rubner (1902) and Scholander *et al.* (1950) have discussed and made use of the concept of a critical ambient temperature at which an animal's maximum tissue insulation is achieved, and below which the metabolic rate is increased in order to maintain the body temperature. Burton & Bazett (1936) have reported detailed results on one man in water which suggested that for man, too, there was a critical temperature, 33° C in water for their subject, at which he achieved his maximal tissue insulation and below which his metabolic rate rose. The fat thickness of this man was not recorded. In the present experiments only the thinnest men behaved approximately in this way, while for the fat men there was a wide difference between the water temperatures at which their metabolic rate increased (about 33° C) and at which they achieved their maximum tissue insulation (about 12° C). The ambient water temperature below which there is an increase in the metabolic rate of men in a steady state of heat exchange, as defined in these experiments, and for immersions of 1–3 hr, may be described as the 'metabolic threshold temperature' in water. It appears to be about 33° C for both fat and thin young men, at least when they are acclimatized to a temperate environment, and to be determined in such men mainly by cutaneous receptors. The lowest water temperature in which a man could in theory achieve thermal stability without an increase in metabolic rate may be described as the 'theoretical critical temperature' and can be calculated approximately for the present subjects from the individual's lowest metabolic rate, his maximum recorded tissue insulation, and his rectal temperature in water at 33° C. It was as high as 32° C. in one thin man (subject 2) and as low as 22° C in a fat man (subject 8). However, the men would presumably have achieved higher insulations in cold water if they had not shivered, so that these calculations of theoretical critical

temperature probably give values that are too high, particularly in the case of the fat men.

The theoretical critical temperature of the present men in air would presumably have been rather lower as a result of the additional insulation provided by the air. Direct evidence about the existence of a critical temperature for naked man in air is somewhat confusing, probably because of the difficulty in obtaining a steady state of heat exchange in air. Men have been reported to shiver in cold air only when their skin temperature falls below about 19° C (Swift, 1932); but Hardy & DuBois (1938) reported that during long exposure naked men showed bursts of shivering in air below about 26° C, while Hardy & DuBois (1940) reported that naked men and women achieved their maximal tissue insulation in still air at 27–29° C.

The critical temperature of naked man in air has also been reported as 28° C on the grounds that men preferred to work rather than to keep still in air below this temperature (Erikson, Krog, Andersen & Scholander, 1956); however, this observation may merely indicate that at this ambient temperature stimulation of the cutaneous cold receptors was sufficient to cause discomfort. Scholander *et al.* (1950) accepting the concept of a critical temperature for naked man and regarding it as 27–29° C in air, considered that this high critical temperature placed man as a tropical animal. The present results suggest that if theoretical critical temperature is used as a criterion this view is an over-simplification.

Effective stimulus to the cutaneous thermoreceptors

Although suggestions, differing in detail, have been made at various times that the thermoreceptors of the body might respond to spatial temperature gradients in the tissues rather than to the level of their local temperature, Hensel & Zotterman (1951) and Hensel & Witt (1959) have shown clearly that the effective stimulus to cold receptors in the tongue was a fall in local temperature. They also review evidence which on balance makes it likely that other superficial thermoreceptors behave in the same way. The present results and those of Keatinge (1960*a*) fit in well with this view: although they do not rule out the possibility that the cutaneous cold receptors might be stimulated in part by spatial temperature gradients as well as by the level of their absolute temperature, they are adequately explained by the hypothesis that both superficial and deep receptors respond principally to the level of their local temperatures, with the superficial receptors showing a limited degree of adaptation to prolonged cold.

Nature of the metabolic response to cold

There is evidence, recently reviewed by Chatonnet (1959), that the metabolic response of adult man to cold is largely brought about by muscular

contraction, although this is not the case in cold-acclimatized rats (Cottle & Carlson, 1956). In the present experiments substantial increases of metabolic rate in cold water were accompanied by shivering whose intensity corresponded roughly with the size of the increase in metabolic rate, and it seems probable that the metabolic response to cold in them was very largely brought about by muscular activity.

Rise in metabolic rate at high temperatures

The fact that the men's metabolic rates were higher in water at 38 than at 34° C is in keeping with many previous observations that the metabolic rate may rise when the body temperature rises (e.g. Houghton, Teague, Miller & Yant, 1929). The rise is generally attributed to a higher rate of heat production by individual tissues at the higher temperature. Although this direct effect of temperature on the tissues must play a part, restlessness in the warmer water may also have been important and so also may increased cardiac work due to cutaneous vasodilatation at the higher temperatures.

Blood flow and subcutaneous fat in regulation of heat loss

The fact that fat men could reduce their loss of heat more than thin men in the cold is also in keeping with earlier reports (Winslow, Herrington & Gagge, 1937; Pugh & Edholm, 1955; Baker & Daniels, 1955–6; Carlson *et al.* 1958; Keatinge, 1960*a*). In particular, these experiments confirm the large increase reported by Carlson *et al.* (1958) in the tissue insulation of fat as opposed to thin men as the bath temperature was lowered. The calculations of Carlson *et al.* were based on brief experimental immersions in water, which probably accounts for certain differences between their results and the steady-state values obtained in the present experiments.

The minimum tissue insulation of fat and thin men was about the same. This observation differs from the report of Winslow *et al.* (1937) that a fat man maintained a higher insulation than a thin man in warm as well as cold surroundings, but is consistent with the observation of Miller & Blyth (1958) that fat and thin men showed a similar rise in rectal temperature when they were exposed to external heat or to exercise. It implies that when the fat men were warm they were able virtually to eliminate the insulating effect of their fat by a high cutaneous blood flow. The rate of heat loss in water at 38° C was as great from the extremities as from the trunk in all men in whom it was measured. At water temperatures of about 33° C, however, heat loss from the fingers was very low, and vasoconstriction in them was presumably almost complete in both fat and thin men. Vasoconstriction must, however, have been incomplete elsewhere in the body

at this temperature, at least in fat men, since they were able to increase their over-all insulation much further at lower water temperatures.

The over-all tissue insulation of the thin men reached a maximum in water not much colder than 33° C and always fell a little when the water temperature was lowered further. Similar falls were reported by Burton & Bazett (1936) and Carlson *et al.* (1958) and are attributed to an increased muscle blood flow due to shivering. The fact that in the present experiments thermal conductivity between the rectum and the forearm skin was generally higher in water at 22 than at 31–33° C, while thermal conductivity to the skin of the trunk was generally the same or lower at the lower water temperature, suggests that this fall in total insulation was largely due to an increased blood flow in limb rather than trunk muscle. The thickness of subcutaneous fat is known to be less in the limbs than the trunk (Edwards, Hammond, Healey, Tanner & Whitehouse, 1955); and Table 1 shows that this was so as regards the arms of the present subjects.

Cold vasodilatation

Although the over-all insulations of the two fattest men rose to high values as the water temperature was lowered to 12° C, they fell sharply below this temperature. These falls in tissue insulation below 12° C were considerably larger than the falls associated with shivering in the thin men at higher temperatures. Since they were associated with a marked and cyclical increase in heat elimination from the finger they were presumably due mainly to cold vasodilatation. It has been shown previously that cold vasodilatation can take place in the fingers of even generally chilled people (Keatinge, 1957) and is due largely to the direct effect of low temperatures on blood vessels (Keatinge, 1958). The dilatation is not confined to the extremities (Clarke, Hellon & Lind, 1958). The present results show that it can greatly reduce the insulation of fat men in water below about 12° C and they imply that no amount of subcutaneous fat would enable these men to survive in near-freezing water for an indefinite period without external protection. In this they appear to differ from aquatic arctic mammals such as whales and seals, which can certainly survive in near-freezing water for long periods.

Effect of physical exertion on heat loss in cold water

Work in water at 16° C (Pugh & Edholm, 1955) and at 5 and 15° C (Keatinge, 1959) accelerates the fall in rectal temperature of thin men but has little effect on that of fat men at these water temperatures or on that of thin men in water at 25° C. The present experiments show that when either fat or thin men were just unable to stabilize their temperatures when sitting still in water, physical exertion always accelerated their falls in

temperature. As suggested previously, this effect of exertion is presumably due to an increased blood flow in muscle, particularly limb muscle. It seems unlikely that physical exertion can ever assist a man to achieve thermal stability during prolonged immersion in water.

SUMMARY

1. The metabolic rate of both fat and thin young men in heat balance in water rose when the bath temperature was lowered below 33° C, although the fat men did not achieve their maximal tissue insulation until the water temperature was much lower. The commonly used concept of 'critical temperature' was therefore not valid in the case of the fat men and alternative terms are proposed.

2. The metabolic rate rose less in fat than in thin men when the bath temperature was lowered below 33° C; the stable rectal temperature of the thin men was lower in cold than in warm water, while that of the fattest men was not.

3. It is concluded that the fat men's small metabolic response to cold was due to reflexes from the skin, while in the thin men these were reinforced by a fall in deep temperature and stimulation of deep temperature receptors.

4. The fat men achieved a higher maximal tissue insulation than thin men and could stabilize their body temperature in water down to 10–12° C. In colder water heat loss from their fingers rose in a cyclical manner, their tissue insulation fell by about 50% and their rectal temperatures fell.

5. Work accelerated the fall in rectal temperature of both fat and thin men in water just too cold for them to stabilize their rectal temperature when still.

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MAXIMUM ACTIVATION OF THE CONTRACTILE MECHANISM IN FROG'S SKELETAL MUSCLE BY POTASSIUM DEPOLARIZATION

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Although there is little doubt that an action potential normally precedes and initiates a contraction of skeletal muscle, there is still some question as to which of the electrical events associated with the action potential is responsible for initiating the contraction. A considerable body of evidence indicates that depolarization of the muscle fibre surface is the responsible electrical event (Biedermann, 1896; Kuffler, 1946; Sandow, 1952; Sten-Knudsen, 1954, 1960), However, some authors have considered the longitudinal current which accompanies the action potential and flows through the myoplasm parallel to the cell surface as the essential event (Bay, Goodall & Szent-Gyorgyi, 1953).

Recently Csapo & Suzuki (1958) have proposed a composite hypothesis. While conceding that depolarization is essential, they propose that longitudinal currents are needed to produce a maximum activation of the contractile mechanism. They further suggest that the lack of longitudinal current could explain the well known observation (Gasser, 1930) that the maximum tension of a contracture produced by placing a frog's sartorius muscle in an isotonic KCl solution is always considerably less than the maximum tetanic tension of the same muscle.

However, there is another possible explanation for the difference in the maximum tensions obtained by the two procedures. Owing to the limited duration of the potassium-induced contracture and the dimensions of the sartorius muscle, it is possible that each muscle fibre is maximally activated when the muscle is placed in an isotonic KCl solution but that the fibres at the surface of the muscle are relaxing before those closer to the centre have begun to develop tension. This would result in a lowered maximum tension of the entire muscle during a potassium-induced contracture. The experiments presented below were designed to test the validity of this explanation.

METHODS

Either the sartorius or the extensor longus digiti IV muscle of the frog, *Rana pipiens*, was used in all experiments. The ext. long. dig. IV, toe muscle, was removed from the frog and dissected free from its sheath and surrounding muscles with the aid of a dissecting microscope. The sartorius was removed from the frog with a portion of the pelvic girdle containing the proximal attachment of the muscle.

The muscles were mounted vertically in a bath which was drained by means of an opening at the bottom and filled from above with a syringe. The lower end of the muscle was fixed near the bottom of the bath and the upper end was attached by means of a nylon thread to the end of a thin brass plate lever approximately 3 in. (76 mm) above the top of the bath. The resting tension on the muscle was adjusted to be just sufficient to maintain the muscle in a vertical position with fluid in the bath.

Electrical stimuli were applied to the muscles either by means of two platinum electrodes 3 mm apart near one end of the muscle, or by two electrodes, one placed at the bottom of the bath and the other at the top of the fluid in the bath. In either case the muscle was completely immersed in solution during stimulation. Supramaximal rectangular pulses, 1–5 msec in duration, were used and the frequency of stimulation was adjusted to obtain a maximum tetanic response of the muscle (60–120 pulses/sec).

The tension produced by the muscles was recorded by means of two strain gauges (SP-4 Type A-7, Baldwin-Lima-Hamilton Corp.) mounted on each side of the lever near its fulcrum. These strain gauges formed two series arms of a Wheatstone bridge. All recordings were made with a Grass model P5 polygraph through a model 5P1 low-level d.c. preamplifier.

Potential changes were recorded by placing a toe muscle in a two-compartment bath. The compartments were isolated by means of an 8 mm petroleum jelly gap surrounding the muscle (see Frank, 1958, Fig. 3). Both compartments contained approximately equal lengths of muscle. The potential difference between the lengths of muscle in the two compartments was altered by changing the ionic composition of the solution in one of the compartments.

In a few experiments the sartorius muscle was reduced in size by successive removal of muscle fibres from the surface and the response of the muscle to isotonic KCl and tetanic stimulation was tested at each stage. The dissections were performed with the aid of a dissecting microscope, care being taken to prevent or minimize damage to muscle fibres remaining in the bundle. An attempt was made to remove fibres so that the remaining bundle of fibres approximated the shape of a cylinder and thereafter to remove fibres uniformly from all surfaces of the bundle. Because of the irregular shape of the sartorius muscle, the weight was used to measure the size of the bundle of muscle fibres at the various stages.

The solutions used had the following composition (mM): NaCl, 111·8; KCl, 2·47; $CaCl_2$, 1·08; NaH_2PO_4, 0·087; $NaHCO_3$, 2·38; glucose, 11·1. The isotonic KCl solution contained 123 mM KCl only or 123 mM KCl + 1·03 mM $CaCl_2$. Solutions with various K^+ concentrations were made by adding appropriate amounts of the isotonic KCl solution to a solution of the above composition, except that the NaCl was replaced by an equimolar quantity of choline chloride. Sufficient D-tubocurarine chloride (Burroughs Wellcome) was added to all the solutions to make a final concentration of 10^{-4} g/ml. During some of the experiments a gas mixture of 0·5 % CO_2 + 99·5 % O_2 was bubbled through the solution. (Solution pH had changed from 7·4 to 7·2 after 30 min exposure to this gas mixture.) All experiments were conducted at room temperature, 20–24° C.

RESULTS

As previously reported (Gasser, 1930), it was observed that the maximum tension produced by placing a frog's sartorius muscle in an isotonic KCl solution was considerably less than the maximum tetanic contraction

of the same muscle. This is illustrated by the upper two tracings in Fig. 1. However, when the same comparison was made with the ext. long. dig. IV muscle, the maximum tension of the potassium-induced contracture was usually 10–20 % greater than the maximum tetanic tension of the same muscle (Fig. 1, lower tracings). The most obvious difference in these two muscles is their size. The diameter of the toe muscle is generally less than 0·5 mm, whereas the cross-sectional dimensions of the sartorius are generally 2–4 mm, by 0·5–1·5 mm.

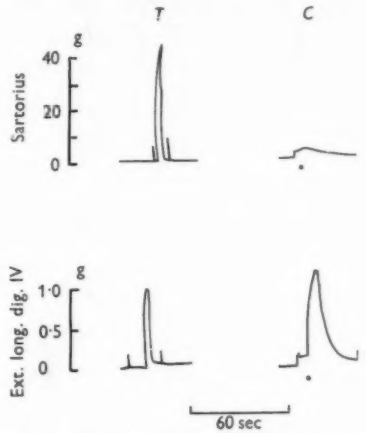

Fig. 1. Maximum tetanic contractions (*T*) and contractures (*C*) produced by isotonic KCl in two muscles of the frog. A single sartorius and single ext. long. dig. IV muscle were used to obtain each pair of responses. Each tetanus is preceded and followed by a twitch. The step at the start of each contracture is an artifact produced by changing the solution in the bath. The dot below each contracture record indicates the point at which the isotonic KCl was put in the bath.

The effects of altering the potassium concentration of the test solution on the potassium-induced contracture and depolarization in the frog's toe muscle are shown in Fig. 2. The depolarization and tension records were taken from separate experiments on two different muscles. As expected, both the degree of depolarization and the maximum tension of the contracture decreased as the potassium concentration of the test solution was reduced. However, as the potassium concentration was reduced the duration of the contracture was prolonged. It is well known that there is a threshold concentration for the potassium-induced contracture (Gasser, 1930). It is possible that the duration of the contracture in the toe muscle is determined both by the duration of the contracture in the individual muscle fibres and by the time needed for the potassium concentration surrounding muscle fibres at various depths in the muscle to reach threshold.

The effects of diffusion on the duration of the potassium-induced contracture can be more clearly demonstrated by reducing the size of a sartorius muscle by the successive removal of muscle fibres from the surface and testing the muscle with isotonic KCl at each stage. When this was done, it was found that the maximum tension of the contracture was only slightly reduced and that the duration of the contracture remained virtually unchanged until the diameter of the remaining bundle of fibres was reduced to about 0·5 mm. At this stage there was an abrupt decrease in both the maximum tension and the duration of the contracture. A further reduction in the size of the muscle fibre bundle reduced the maximum tension but not the duration of the contracture.

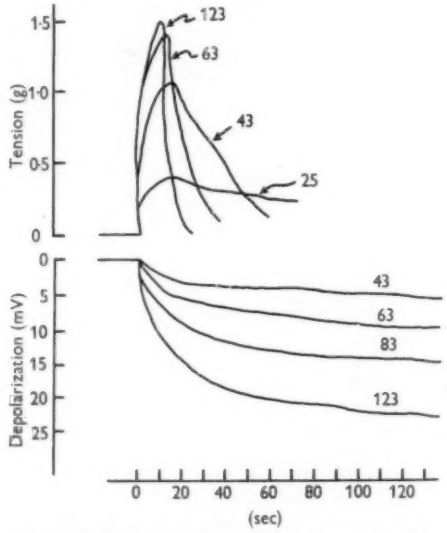

Fig. 2. Contractures and depolarizations of the ext. long. dig. IV muscle produced by placing the muscles in solutions of various potassium concentrations: numbers show mM concentrations. The tension and depolarization records were obtained in separate experiments on different muscles.

If, in this experiment, the maximum tensions of contracture and of tetanus are compared at each stage (Fig. 3), it can be seen that the difference between the two values decreases abruptly as the size of the remaining bundle of muscle fibres approaches that of the toe muscle. At the latter stage the maximum tension of the contracture is at least as great as that of the tetanus.

In the experiments illustrated in Fig. 3 it was assumed that the maximum tetanic tension was a measure of the maximum possible response of the

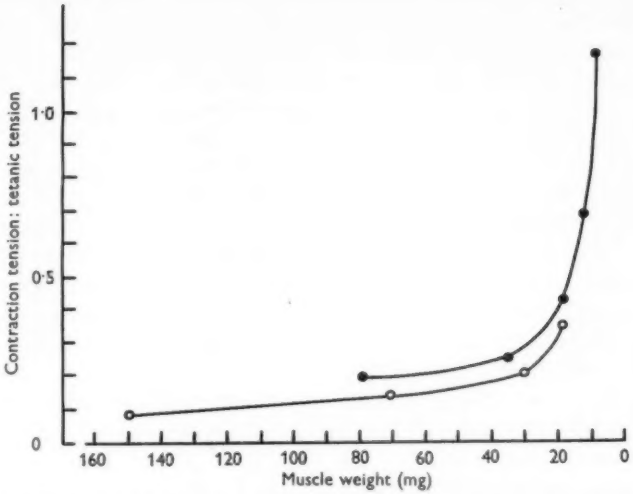

Fig. 3. Change in the ratio of the maximum tension of a potassium-induced contracture to the maximum tetanic tension in the sartorius, resulting from a reduction in the size of the muscle by removing fibres from the outer surface of the muscle in successive stages. ○ and ● represent results obtained from two separate experiments.

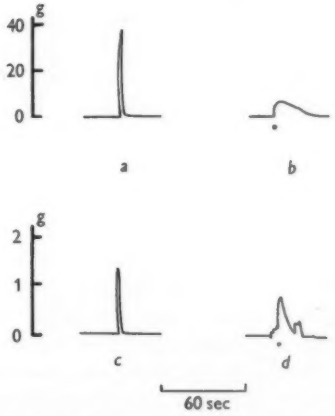

Fig. 4. Effects of reducing the cross-sectional area of a sartorius muscle on maximum tetanic contractions (*a* and *c*) and contractures produced by isotonic KCl (*b* and *d*). *a* and *b*, responses of intact muscle; *c* and *d*, responses following dissection. The dot below each contracture record indicates the point at which isotonic KCl was put in the bath. For details see text.

muscle under the prevailing conditions. However, if special pains are taken it is possible to get maximum tetanic tensions (in kg/cm²) which are not significantly changed during the experiment. These special precautions include the use of oxygen, a minimal exposure to isotonic KCl, a rapid, careful dissection and a careful adjustment of the muscle length. In the experiment of Fig. 4, the wet weight of the whole muscle (Fig. 4*a* and *b*) was 51 mg, and after dissection (Fig. 4*c* and *d*) the remaining muscle weighed 2·5 mg. In both cases the length was about 40 mm. From these figures and the tensions recorded (Fig. 4*a* and *c*) it was calculated, by tension × length/weight, that the maximum tetanic tension of the intact muscle was 2·29 kg/cm² and after dissection it was 2·21 kg/cm². The calculated cross-sectional area of the dissected muscle was $0{\cdot}63 \times 10^{-3}$ cm². By direct measurement the dissected muscle was found to have a cross-sectional area of $0{\cdot}64 \times 10^{-3}$ cm² at its narrowest point and $1{\cdot}22 \times 10^{-3}$ cm² at its widest point. The contracture tension:tetanic tension ratio of the intact muscle was 0·21 and after dissection it was 0·58.

DISCUSSION

A considerable body of evidence dating from the double myograph studies described by Biedermann (1896) to the recent micro-electrode studies of Hagiwara & Watanabe (1955) shows that depolarizing a skeletal muscle will lead to a mechanical response, whereas longitudinal currents will not. The question considered here is whether depolarization *per se* is sufficient for maximum activation of the contractile mechanism of a skeletal muscle fibre. The only indication that it might not be sufficient is the difference in the maximum tensions of the potassium-induced contracture and the tetanus in skeletal muscles the size of the frog sartorius and larger (Csapo & Suzuki, 1958). However, the fibres of the toe muscle are apparently maximally activated by an isotonic KCl solution (Fig. 1; see also Frank, 1960), and it would seem reasonable to assume that the same holds true for the fibres of the sartorius muscle. Therefore the reduced size of the potassium-induced contracture in the sartorius muscle appears to be a diffusion phenomenon in which the fibres at the surface relax before the potassium concentration surrounding fibres at the centre of the muscle is large enough to produce a maximum activation of the latter fibres. In accord with this explanation, the difference in the two maximum tensions in the sartorius muscle is eliminated when the diffusion distance for potassium ions is sufficiently reduced (Fig. 3), and it also has been reported (Horowicz & Hodgkin, 1956) that the contracture tension of an isolated semitendinosus muscle fibre is about equal to the maximum tetanic tension.

The larger magnitude of the maximum tension of the potassium-induced contracture in the toe muscle was observed in seven out of nine experiments

and in the other two experiments the differences in the maximum contracture tension and maximum tetanic tensions of the same muscle were small. It is therefore surprising that Csapo & Suzuki (1958) failed to make this observation. One possible explanation of the different observations in their work and in the work reported here is that they might have used toe muscles having larger cross-sectional dimensions than those used in the experiments reported here. Their use of larger muscles is indicated by the fact that the tensions produced by the toe muscles in their experiments are larger than those usually observed in the work reported here and they rejected toe muscles which developed maximum tensions less than 4 g. The cross-sectional areas of all the toe muscles used in the present investigation were between 0·04 and 0·14 mm^2. Assuming that the toe muscle is capable of developing a tetanic tension equivalent to the sartorius, e.g. 2·5 kg/cm^2 (Hajdu, 1951), the theoretical maximum tension for the largest toe muscle used here would be 3·5 g. They also report an experiment similar to the one illustrated in Fig. 3. However, from their results it appears that they did not sufficiently reduce the size of their muscle bundle and therefore they observed only the small change in the ratio shown in the lower part of the curve in Fig. 3.

As was mentioned above, the maximum tension of the potassium-induced contracture in the toe muscle was usually greater than the maximum tetanic tension of the same muscle. This difference was probably due to the presence of an appreciable number of slow muscle fibres (Kuffler & Vaughan Williams, 1953; Gray, 1958). Since these fibres do not have propagated action potentials it is likely that they would not be maximally activated by direct muscle stimulation in the presence of tubocurarine. Indeed, it was often possible to demonstrate a contracture lasting several minutes by placing a toe muscle in a solution containing acetylcholine. The tension of this maintained contracture was at most some 20 % of the maximum tetanic contraction and could account for the difference between the potassium-induced contracture tension and the tetanic tension in the same muscle. It is interesting that Gray (1958) found by anatomical observation that 6–20 % of the fibres in the toe muscle of the frog, *Rana temporaria*, were slow muscle fibres.

It has long been known that depolarization is not the only way in which the contractile mechanism of a skeletal muscle fibre can be activated (Gasser, 1930). There is also evidence that the degree of activation is not directly tied to the degree of depolarization. For example, in Fig. 2 above it is seen that contracture is of a limited duration despite the continued presence of a depolarization when the toe muscle is kept in a solution having an elevated potassium concentration. The lack of an inflexible relation between depolarization and tension development has been inter-

preted by Shanes (1958) as suggesting that some other process might be more directly responsible for activating the contractile mechanism. However, the above demonstration that a depolarization by itself is sufficient to cause a maximal activation of the contractile mechanism of a skeletal muscle, strongly supports the contention of previous workers that the depolarization associated with the action potential is the responsible electrical event.

SUMMARY

1. It was observed that the maximum tension of a contracture produced by placing the frog's extensor longus digiti IV muscle in an isotonic KCl solution was usually at least as large as the maximum tetanic tension of the same muscle. In the intact sartorius muscle the maximum tetanic tension is always greater.

2. When the sartorius muscle was reduced in stages by the successive removal of fibres from the outer surface of the muscle, it was found that the difference in the maximum tension of the potassium-induced contracture and the tetanus was reduced and eventually eliminated.

3. It is suggested that the smaller size of the maximum tension of the potassium-induced contracture in muscles as large as the frog sartorius and larger is due to a diffusion phenomenon in which the fibres at the surface of the muscle have begun to relax before the potassium concentration in the extracellular fluid surrounding fibres at the centre is large enough to initiate a mechanical response.

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CORRELATION OF THE INHIBITORY POST-SYNAPTIC POTENTIAL OF MOTONEURONES WITH THE LATENCY AND TIME COURSE OF INHIBITION OF MONOSYNAPTIC REFLEXES

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Afferent volleys in the second or third sacral dorsal roots inhibit the contralateral motoneurones with a central latency which is so brief that Lloyd & Wilson (1959) have been led to draw several conclusions, which they have extended to the so-called direct or Ia inhibition as investigated with volleys in limb nerves (cf. Lloyd, 1960): that the central inhibitory path is monosynaptic; that the inhibitory post-synaptic potential of motoneurones is not the primary agent of this inhibition, but may be a secondary and later manifestation of the inhibition; that the spike potential recorded intracellularly in the soma cannot be used as an index of the reflex discharge of an impulse along the axon.

These conclusions stand as a challenge to an extensive conceptual structure that has been built upon a precise study of Ia inhibition both by intracellular recording from motoneurones, and by a careful correlation of these results with inhibition of monosynaptic reflexes discharged by the motoneurones (Eccles, 1953, 1957, 1958; Coombs, Eccles & Fatt, 1955*b*; Curtis, Krnjević & Miledi, 1958; Frank & Sprague, 1959; Curtis & Eccles, 1959). Thus it has been concluded that the inhibitory action of a Ia afferent volley is fully explained by the motoneuronal hyperpolarization produced by that volley (the inhibitory post-synaptic potential, IPSP), or more strictly by the IPSP together with the ionic currents that generate it. It has further been concluded, from an extensive series of investigations, that the central pathway for Ia or 'direct' inhibition is disynaptic, and the inhibitory interneurones on this path have been located and shown to have the requisite properties (Eccles, Fatt & Landgren, 1954, 1956; Jack, 1957; Curtis *et al.* 1958; R. M. Eccles & Lundberg, 1958). Finally, it has

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been shown that the IS component of the spike potential, as recorded intracellularly, is a direct antecedent of the impulse discharged along the axon in the ventral root (Araki & Otani, 1955; Fuortes, Frank & Becker, 1957; Coombs, Curtis & Eccles, 1957*a*, *b*).

Evidently the contradiction extends to the very nature of synaptic action on neurones (cf. Lloyd, 1960), and its resolution is hence of urgency and importance. Crucial experiments have been designed for this purpose. Lloyd & Wilson (1959) make the point that it is essential to investigate the inhibition of impulses discharged along axons in the ventral root, rather than impulses recorded in the somas of motoneurones. The present investigation has conformed to this criterion, the latency of Ia inhibition being measured directly on the monosynaptic reflex spike, which has never previously been attempted except for one record published by Frank & Sprague (1959). These latencies and a series of other investigations establish that the IPSP accounts precisely for Ia inhibition of monosynaptic reflexes. In the discussion it is argued that the conclusions of Lloyd & Wilson (1959) are refuted, and that the contradiction is thereby resolved.

METHODS

The animals (ten cats) were under light Nembutal (pentobarbitone; Abbott Laboratories) anaesthesia with the spinal cord divided at the second lumbar segment. For the investigation of Ia inhibition of motoneurones supplying hind-limb muscles, nerves to quadriceps (Q) and to posterior biceps-semitendinosus (PBST) muscles were mounted on stimulating electrodes, and the ventral roots from L5 to S1 were severed. The S1 or the lower half of the L7 ventral root was mounted for monophasic recording of reflex spikes produced by Group I PBST volleys. In order to define accurately the onset of inhibition as illustrated in Figs. 1–3, it was essential to have a large and relatively stable reflex spike potential. In most experiments facilitation or potentiation of the monosynaptic reflex was required for this purpose. This was achieved either by facilitation by a reflexly subliminal PBST volley about 1·5 msec earlier, or by potentiation by a brief tetanus (450/sec for 200 msec) of the PBST nerve ending about 150 msec earlier. This latter procedure was preferable and was adopted in our later experiments, the interval between successive tests being lengthened to 7 sec instead of the 3·5 sec which was employed when single or double stimuli were applied to the PBST nerve. If the latency of inhibition is to be demonstrated by the reflex spike responses, the interval between the inhibitory (Q) volley and the excitatory (PBST) volley must be chosen so that the onset of the inhibition occurs during the rising phase of the reflex spike potential (cf. Figs. 1–3). Furthermore, the sweep speed must be so fast that the superimposed traces of the inhibited spikes and the control spikes are clearly distinguishable. This was accomplished by expanding an appropriate part of the sweep so that it covered the whole face of the tube. When the spike was potentiated as above, the sweep was accelerated about 200 times so as to give records such as those of Figs. 1A–F, 3. Similar procedures were employed when investigating the inhibitory action of the contralateral S3 afferent volley on the monosynaptic reflex spikes which an ipsilateral S3 afferent volley evokes in the equivalent ventral roots. The afferent volleys were always recorded by an electrode on the appropriate dorsal root at its entry into the spinal cord, such electrodes being at the upper L6 and S1 levels for Q and PBST volleys respectively. The arrival time of the volley at the cord was taken to be the peak of the first (positive) deflexion, as indicated in Figs. 1–3.

When recording potentials electrotonically transmitted along the ventral roots, the spinal cord was rotated by traction on the denticulate ligament and slightly raised from its bed by small balls of cotton wool. In order to discover and utilize the ventral-root filaments containing the highest proportion of axons of PBST motoneurones, the S1 or lower L7 ventral roots were carefully split into filaments which were mounted one at a time on fine platinum hooks employed as recording electrodes. The proximal electrode was within 1 mm of the exit of the root, while the distal was at least 15 mm away. Root splitting of large ventral roots had the additional advantage that it made it easier to record from the ventral-root fibres as soon as they emerged from the spinal cord. It was not as a rule necessary for the much smaller S3 ventral root.

The procedure for intracellular recording from motoneurones has already been described (Brock, Coombs & Eccles, 1952; Coombs *et al.* 1957*a*). Since IPSPs were being recorded, the electrodes were filled with 0·6 M-K_2SO_4 or 2 M-K citrate solution. The excitability of motoneurones was tested during IPSPs by means of square pulses applied through the intracellular micro-electrode in the depolarizing direction (cf. Fuortes *et al.* 1957; Coombs, Curtis & Eccles, 1959).

RESULTS

The central latency of Ia inhibitory action on motoneurones

It has been generally accepted that a comparison between the central latencies of Ia inhibitory action and of Ia monosynaptic excitatory action cannot simply be derived from an investigation of the latest time at which an inhibitory volley can be placed relative to an excitatory and yet exert an inhibitory action on the height of the reflex spike potential as recorded in a ventral root. Even under the most favourable conditions the monosynaptic reflex discharge is composed of individual discharges dispersed over a considerable fraction of a millisecond, as is indicated diagrammatically in Fig. 1I, J. With such a critical disposition of the volleys the inhibition would be exerted on the latest reflex discharges that contributed to the summit height of the spike potential, as indicated diagrammatically in Fig. 1K, L (cf. Eccles *et al.* 1954, 1956; Frank & Sprague, 1959; Lloyd & Wilson, 1959); yet this presumed effect has not been investigated, except in one illustration by Frank & Sprague (1959), who, however, did not utilize it in measuring the latency of central inhibitory action.

In Fig. 1A–F the inhibitory Ia volley in quadriceps (Q) nerve preceded the excitatory PBST volley by intervals ranging from 0·5 to 1·0 msec, as measured from the traces recorded by an electrode on the dorsal root at the upper L6 segmental level. A second electrode at the entry of the upper S1 dorsal root revealed that the BST volley entered the spinal cord 0·17 msec earlier than its arrival at the L6 level, as indicated by the electrical potential it generated there. The records have been arranged so that the spike potentials of the Q volleys are in the same vertical line (the dotted line to the left). Each record was formed by the superposition of about eight traces of the inhibited reflex spike and eight traces of the uninhibited reflex spike. It will be noted that the traces of reflex spikes in

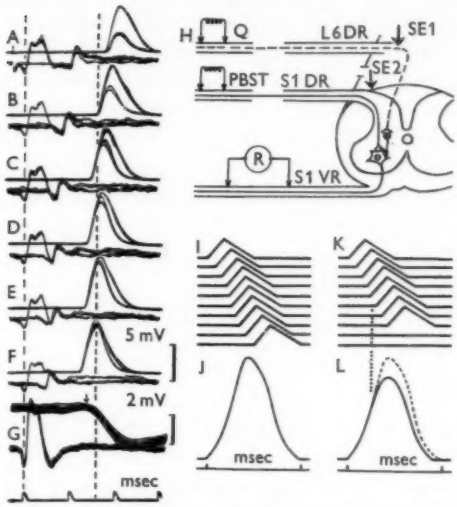

Fig. 1. The experimental arrangements are shown diagrammatically in H. In A–F a monosynaptic reflex spike response (monophasically recorded from S 1 ventral root (upper traces)) was generated by an afferent volley from PBST, which also produced a diphasic spike in the records from the dorsum of the cord (lead SE 1 in H) at the upper L 6 level (lower traces). This PBST volley reached the cord at various times after a maximum Group I afferent volley from quadriceps, which is at a fixed position in A–F, its arrival time at the upper L 6 level being given by the left perpendicular broken line. The superimposed traces for each of the testing intervals A–L were formed by first photographing eight traces for the BST volley alone at 7 sec interval to give the control reflex spike, and then a further eight traces with quadriceps volley in addition. It will be seen that at each testing interval the inhibited reflex spike deviates from the control. The second vertical broken line at 1·59 msec from the first passes through the onset of the inhibition so signalled by the reflex spikes for all but record A, where evidently the inhibition was operative before the beginning of the testing reflex response. In G there is an IPSP produced by the Q volley and intracellularly recorded from a PBST motoneurone at the S 1 segmental level a little later in this same experiment. The record of the Q volley at the upper L 6 segment was at higher amplification, but otherwise very similar, and it is exactly synchronized with the Q volleys in A–F, the sweep speeds being identical. Note different potential scales for the reflex spike and for the IPSP. The manner of production of a record such as C or D is illustrated in the constructions, I–L. In I there are schematic reflex spikes in ten fibres dispersed over 0·5 msec, as in a normal monosynaptic reflex, which is derived as in J by summation. Inhibition, beginning at the dotted line in K, delays the onset of the 6th to 8th spikes, as shown, and suppresses the 9th and 10th. As a consequence the summed reflex potential (the continuous line in L) deviates from the control (the broken line in L) at a point just later than the dotted line.

Fig. 1C closely resemble the construction in Fig. 1L. As would be expected in the several records at the different test intervals the onset of inhibitory depression of the reflex spike occurred at virtually the same time after the inhibitory volley, the respective points of divergence lying very close to the perpendicular broken line 1·59 msec later than the line giving the arrival time of the inhibitory volley at the upper L6 segment. In Fig. 1A the inhibition had already begun before the onset of the testing spike.

An alternative method for measuring central latency (Fig. 2) has the advantage of giving a reflex spike potential that is more expanded in

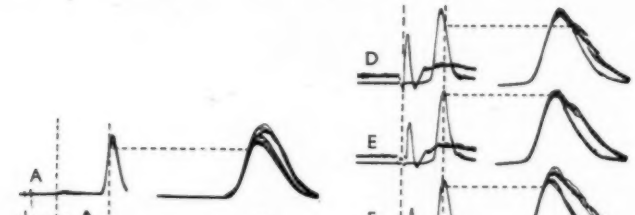

Fig. 2. A–C; PBST monosynaptic reflex spike response in S1 ventral root and its inhibition by the quadriceps volley as in Fig. 1, but in a different experiment. D–H; ipsilateral monosynaptic spike response in S3 ventral root and its inhibition by the contralateral S3 dorsal-root volley. In each set of records the left traces show the inhibited reflex spike (upper beams) and the afferent volley recorded on the dorsal-root entry (lower beams). The inhibitory volleys are at a fixed position in the sweep and the excitatory volleys are shifted from the left to the right in the series of A to C and D to H. The right-hand traces in each series illustrate the monosynaptic reflex spike with a faster sweep velocity, the inhibited responses (8 traces) being superposed upon the control ones (8 traces). The horizontal broken lines are drawn from the diverging points of the inhibited and control responses in the faster sweep to the corresponding points on the companion slower records, through which the vertical interrupted lines are drawn so as to indicate the onset time of the inhibition, as in Fig. 1. The vertical lines to the left indicate the time of arrival of inhibitory volleys at the spinal cord, at upper L6 level in A–C and at S3 level in D–H. Since the afferent volley was recorded at upper S1 level in A–C, the first vertical line was drawn delayed by 0·13 msec. In I, a specimen IPSP recorded intracellularly from a motoneurone in S3 region is illustrated with the same time base, its onset being marked by a small arrow.

time, so that the first point of divergence of the control and inhibited spike potentials can be more accurately determined than in Fig. 1. From this point horizontal lines are drawn to the companion slower records on which the inhibitory latency is measured as in Fig. 1, and in Fig. 2A–C this latency is 1·82 msec. Since in this series the afferent volleys were recorded at S1 level, an allowance of 0·13 msec was made in drawing the first vertical line in order to allow for the measured propagation time of the Q volley to its entrance into the cord at upper L6 level.

Altogether by the one or other method the inhibitory latency of Q volleys on PBST monosynaptic reflexes has been measured in experimental series in six preparations. The values have ranged from 1·59 to 1·90 msec with a mean of 1·72 msec.

Inhibitory latencies determined as in Figs. 1 and 2A–C can be readily compared with the latencies of the IPSPs produced by Q volleys in PBST motoneurones at the same segmental level. For example, in Fig. 1G there is the IPSP recorded shortly afterwards in a PBST motoneurone. When arranged in this manner the onsets of the IPSPs have been found to precede the beginnings of the reflex spike inhibition by 0·06–0·3 msec (mean 0·21 msec), which corresponds approximately to the conduction time of the reflex spike from the cord to the proximal recording electrode. For example, in the experiment of Fig. 1 there was 0·28 msec between stimulation of the ventral root through the proximal recording electrode and the onset of the antidromic spike potential as recorded in the soma (Coombs *et al.* 1957*a*). Thus the central inhibitory latency as derived from Fig. 1 is 1·31 msec, which is to be compared to 1·37 msec for the latency of the IPSP in Fig. 1G. This excellent agreement between the latencies of the IPSPs of motoneurones and the reflex spike inhibitions has been seen in every experimental series.

Similar investigations on the latencies of reflex spike inhibition and of IPSPs have been performed on the inhibition of motoneurones in the S3 segmental level by volleys in the lowest threshold afferent fibres of the contralateral S3 roots. As is illustrated in Fig. 2D–H and Fig. 3, the latency of inhibition of the reflex spike could be similarly determined and compared with the latency of the IPSP of the same motoneurones. The series of Fig. 2D–H resembles Fig. 2A–C in the method of deriving the inhibitory latency, which was 1·74 msec for the spike, as compared with 1·53 msec for the IPSP in I. In the series of Fig. 3A–D the monosynaptic reflex spikes were facilitated by a submaximal S3 volley 2 msec earlier, as described in Methods. The latency of the inhibitory action of the contralateral S3 volley on the reflex spikes as measured between the two vertical lines was 1·58 msec, while in E and J the latencies of the intracellularly recorded IPSPs, as measured to the arrows, were 1·38 and 1·20 msec,

respectively. In the series F–I on the same preparation the reflex spikes were potentiated by a brief conditioning tetanus, as described in Methods. The contralateral inhibitory action on the very large reflex spike was much reduced, but the great stability of the reflex allowed the latency to be accurately measured, the interval between the two vertical lines giving a value of 1·50 msec.

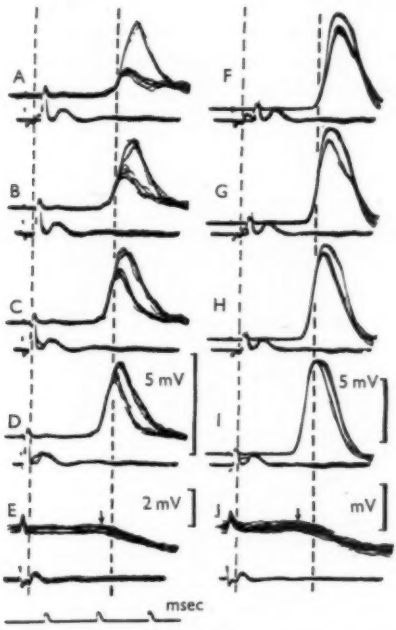

Fig. 3. The latency measurement of inhibition in the S3 ventral root, with two different methods of potentiation of the reflex. Arrangements of records are the same as in Fig. 1. The monosynaptic reflex by the ipsilateral dorsal root stimulation was potentiated in A–D by a subliminal conditioning stimulus and in F–I by tetanic stimuli (see Methods). E and J show IPSPs recorded intracellularly from two different motoneurones in the S3 segment of the same preparation.

The inhibitory latency of reflex spikes in the S3 ventral root was measured in many series in five separate experiments, the mean value being 1·57 msec (range, 1·31–1·78). When allowance was made for the conduction time to the proximal recording electrode on the S1 ventral root (0·2–0·3 msec), there was again excellent agreement between the latencies of the inhibitory action on the reflex spike and of the IPSP, the mean value for the latter being 1·32 msec (range 1·20–1·40).

In contradistinction, Lloyd & Wilson (1959) calculated that the latency

for reflex spike inhibition was as much as 1·0 msec briefer than the latency of the IPSP generated by Ia afferent volleys. It will be shown in the discussion that this discrepancy derives from errors in their assumptions and in the calculations derived therefrom.

Since there is now such excellent agreement between the central latencies for inhibition as tested by the IPSP and by the inhibition of reflex spikes, it is justifiable to use the former measurements when comparing the central latencies for Ia inhibitory and excitatory action on motoneurones, a procedure which has been routinely employed (Brock *et al.* 1952; Eccles *et al.* 1954, 1956; Curtis *et al.* 1958; Frank & Sprague, 1959). In the present experiments the mean central latency for the IPSP was 0·75 msec longer than for the EPSP of S 3 motoneurones and 0·91 msec longer for inhibition of PBST motoneurones. As previously noted (Eccles *et al.* 1956), the latter value is longer because of the longitudinal path of 10–15 mm down the spinal cord. With approximate equality of the inhibitory and excitatory pathways the latency differential is reduced to about 0·75 msec. The presynaptic impulses responsible for the inhibitory action on PBST motoneurones have been recorded in the immediate environment of the inhibited motoneurones only 0·3 msec before the onset of the IPSP (Eccles *et al.* 1956), hence the same time of 0·3 msec is occupied by excitatory and inhibitory presynaptic impulses in initiating the EPSP and the IPSP respectively. The latency differential of about 0·75 msec cannot therefore be attributed either to differences in the times involved in the actual synaptic mechanisms or in the lengths of the respective central pathways. Frank & Sprague (1959) reported that there was a briefer latency differential (range 0·3–0·7 msec) for the EPSPs and IPSPs at the S 2 and S 3 levels, which is briefer than values observed by Curtis *et al.* (1958) and in the present experiments. However, their EPSPs and IPSPs showed a shortening of latency by as much as 0·6 msec as the stimulus strength was increased. In the absence of records from the dorsal roots showing the timing of the volleys entering the spinal cord, the abnormally large latency shifts remain inexplicable, but suggest that the brief latency differentials of 0·3–0·7 msec cannot be regarded as in conflict with the mean differential of about 0·8 msec obtained in all other investigations.

The time course of the Ia inhibitory action

In the standard procedure the time course of the inhibitory action of a single Ia afferent volley has been determined by observing the depression of a testing monosynaptic reflex at various intervals after the inhibiting volley. Inhibitory curves so determined have always had a sharp rising phase, maximum inhibitory action being observed at stimulus intervals of 0·5–1·0 msec, but there have been very large differences in the decaying

phases reported by different investigators. In the first description there was an approximately exponential decay with a time constant of about 4 msec (Lloyd, 1946), but this curve was only rarely obtained by Laporte & Lloyd (1952), who attributed the much more rapid decay that was usually observed to a superimposed disynaptic excitatory action of the Ib afferent impulses which contaminated the conditioning volley. However, Bradley, Easton & Eccles (1953) regularly found this fast initial phase of decay even when the conditioning volley had been shown to have no appreciable admixture of Ib impulses. It was later postulated that the time course of this inhibitory curve was due to two superimposed factors. The hyperpolarization of the IPSP would be expected to give an inhibitory curve with an approximately exponential decay, as observed originally by Lloyd; but superimposed thereon is an initial brief depressant action produced by the intense ionic currents that flow for about 2 msec through the patches of post-synaptic membrane activated by the inhibitory transmitter (Coombs, Eccles & Fatt, 1955*c*; Curtis & Eccles, 1959). Finally, Jack, McIntyre & Somjen (1959 and personal communication) have suggested that, when the spinal cord is in particularly good condition, the membrane potential of the motoneurone is so high that no hyperpolarization is produced by inhibitory synaptic action. The Ia inhibition would thus be due entirely to the ionic currents, and consequently would have a duration of about 2 msec. This extreme position was based on two experimental findings. Occasionally very brief inhibitory curves were observed, there being no detectable inhibitory action after the initial intense phase; and, in contrast to excitatory post-synaptic potentials, no inhibitory post-synaptic potentials could be recorded as a result of electrotonic transmission to the ventral root as it emerged from the spinal cord. The intracellularly recorded IPSP was thus postulated to be a consequence of the lowering of membrane potential due to the impalement by the micro-electrode (cf. Lloyd & Wilson, 1959).

It has therefore been important to redetermine the Ia inhibitory curve under conditions in which complicating excitatory Ib actions could be excluded. Invariably it has been found that the initial intense phase has declined smoothly into a much more slowly decaying component, exactly as described by Bradley *et al.* (1953) and as usually found by Jack *et al.* (1959). For example, in Fig. 4 very weak stimulation of the quadriceps nerve was employed to inhibit the monosynaptic reflex spike evoked by a maximal Group I BST volley in S1 ventral root. The stimuli for the two inhibitory curves plotted in Fig. 4B were 1·2 and 1·4 times threshold, and well below the threshold for Ib afferent fibres, which by the double stimulation technique (Bradley & Eccles, 1953; Eccles, Eccles & Lundberg, 1957*a*) was determined at 1·7 times threshold. The broken lines indicate

for these two inhibitory curves the approximate course of the inhibition that could directly arise on account of the hyperpolarization recorded in the IPSP of Fig. 4A. The inhibitory curve that had the least slow component is shown in Fig. 5G, together with specimen records of the inhibited reflex spike at several testing intervals (Fig. 5A–E), and in Fig. 5F tracings of the quadriceps afferent volley and a specimen IPSP. An unusual procedure was employed in assessing the abscissae in Fig. 5G: instead of the conventional plotting of intervals between the entry of the inhibitory and

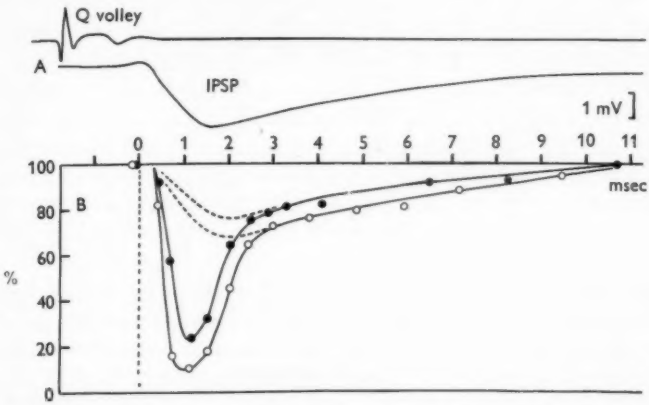

Fig. 4. Inhibitory curve in which the inhibitory action of a quadriceps Ia afferent volley is tested by the size of a monosynaptic reflex spike discharged into the S1 ventral root from BST motoneurones. In B the ordinates show the percentage size of the reflex spike, the abscissae the testing volley intervals. ●, inhibitory stimulus 1·2 threshold, ○ 1·4 threshold for Ia fibres. The dotted lines indicate the approximate time courses of the components of inhibition attributable directly to the hyperpolarization of the IPSP, which is seen in the traced record in A. The afferent quadriceps volley (upper trace of A) is recorded at the upper S1 level.

excitatory volleys into the cord, the abscissae in Fig. 5G are measured from the entry of the inhibitory volley to the summit of the reflex spike potential. As a consequence the latency of origin of the curve (1·8 msec) is the result of approximately the same method of measurement of the latency of inhibition as is given by the interval between the vertical lines in Fig. 1. It is seen likewise to be a little longer than the latency of the IPSP in Fig. 5F.

The manner in which a Ia inhibitory curve is built up from the responses of individual motoneurones is illustrated by testing for the range of intervals over which a motoneurone is inhibited by a Ia inhibitory volley of constant size. If the testing monosynaptic excitation is diminished in size, the range of the inhibited intervals increases as illustrated in Fig. 6. In

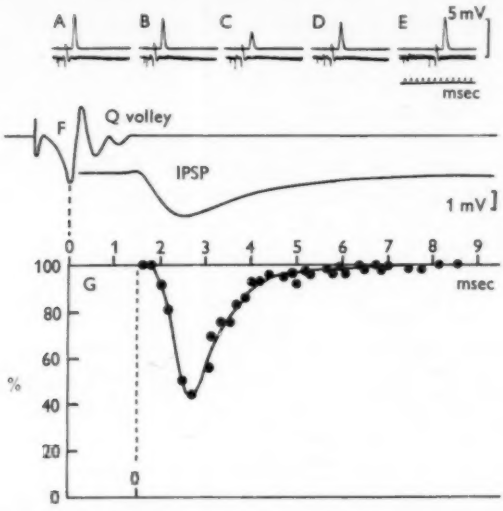

Fig. 5. An example of the inhibitory curve in which the quadriceps volley showed a relatively small later effect upon the PBST monosynaptic reflex (compare with Fig. 4). A–E specimen records of inhibited spikes (upper beams) at five different intervals. The lower beams are potentials recorded on the dorsal surface of the cord at upper S1 level. A specimen IPSP recorded intracellularly from a motoneurone in the same preparation is traced in F together with the record of the quadriceps afferent volley. In G the ordinates give the percentage sizes of the reflex spikes and the abscissae are measured from the arrival of the quadriceps volley to the summit of the reflex spike. The zero point in the time scale is at the arrival of the quadriceps volley at the upper S1 level, its entrance to the upper L6 level being at 0·17 msec later. Zero volley interval, as in Fig. 4B, is shown below.

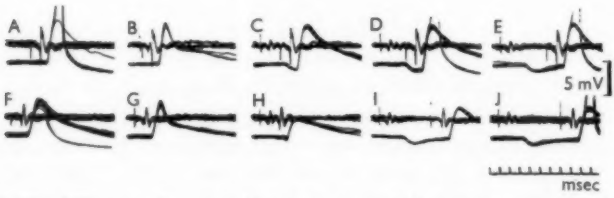

Fig. 6. The difference of ranges of intervals over which a motoneurone, activated by Ia EPSP of different sizes, is inhibited by Ia inhibitory volley of a constant size. The lower traces are potentials intracellularly recorded from a PBST motoneurone and upper ones the afferent volleys recorded at upper S1 level. A–E, test volley maximal for Group Ia; F–J, test volley about 2/3 maximal for Ia.

the series A–E the testing reflex spike was evoked by a maximal Group Ia volley, and as shown in the control (A) it evoked a spike in all but one of about eight superimposed recordings. There was complete inhibition only at the relative short testing intervals of 0·36 and 2·14 msec (B, C), i.e. at the maximum of the inhibitory curve. In the series, F–J, the testing reflex spike was evoked by a Group Ia volley that was only about two thirds maximal, which evoked a reflex discharge in only two of the eight superimposed tracings. There was now complete inhibition with testing intervals ranging from 0·18 msec (G) to 5·3 msec (I).

The disadvantage of testing for inhibition by its diminution of a testing reflex discharge is that the ordinates give the relative population of the discharging motoneurones and do not give a direct measure of the intensity of inhibitory action. Such a measure is provided if the test stimulus is a brief rectangular current pulse that is applied in a depolarizing direction through the intracellular electrode during the IPSP (Frank, 1959; Eccles, 1958). At each testing interval during the inhibition, the pulse was varied in intensity until it evoked a spike potential in approximately one half of the trials (Fig. 7A–H). The spike potential was recorded either by the intracellular electrode, or from the appropriate ventral root filament. Figure 7K shows inhibitory curves obtained by this technique, the ordinates being measured as relative excitabilities, i.e. as reciprocals of the threshold current intensities. When the current pulse was very brief, as in A–D (0·2 msec), the time course of the inhibitory curve (Fig. 7K, short rectangles) corresponded closely with the time course of the IPSP (Fig. 7J). However, when, as in Fig. 7E–H, longer current pulses (0·8 msec) were employed, the same IPSP produced a much larger depression of excitability at the optimum intervals (Fig. 7K, long rectangles), but this brief intense phase was followed by a longer low-intensity phase where the depression was little more than that with the briefer test pulses. There is some uncertainty how such long pulses can be related to a curve that expresses the time course of the inhibitory depression. The whole duration of the pulse is evidently concerned in generating the discharge of an impulse. The best compromise is probably to regard the inhibitory curve as being defined by the mid points of the pulses, as in Fig. 7K. The time course of depression, as revealed by the long pulses, corresponds closely to the inhibitory curve determined by depression of monosynaptic reflexes (Figs. 4, 5). The relationship of these various types of inhibitory curves to the IPSP and the post-synaptic currents that generate it will be considered in the Discussion.

The electrical potential changes produced by Ia inhibitory action

Lloyd & Wilson (1959) and Jack *et al.* (1959 and personal communications) have reported that synaptic inhibitory action on motoneurones was not associated with any electrotonic spread of a hyperpolarization down their axons and so to the ventral root, though excitatory synaptic action on those same motoneurones caused the spread of a large depolarization. However, if the motoneurones were depolarized, the inhibitory action was observed to depress this depolarization, i.e. to produce a relative hyper-

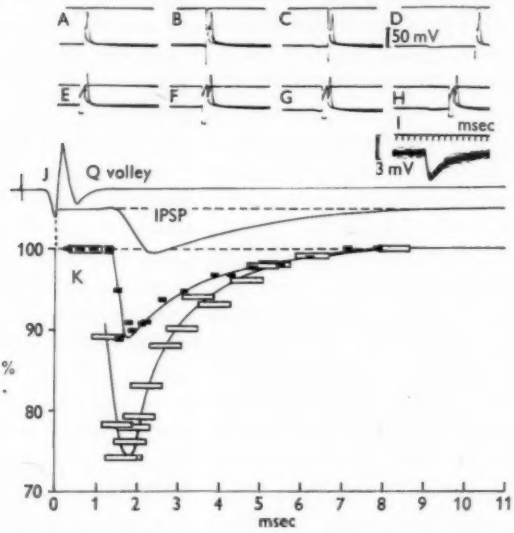

Fig. 7. In A–H depolarizing rectangular current pulses (the upper records with the pulses recorded downwards) were applied through an intracellular electrode (filled with 2M potassium citrate) in a biceps-semitendinosus motoneurone and adjusted in strength so that a spike potential was evoked in about half of the trials, as shown in the lower records, there being about 10 superimposed traces in each record. With A–D and E–H the pulse durations were 0·2 and 0·8 msec respectively and the control threshold current strength was 3·3 times larger in A than in E. In B–D and F–H the pulses tested excitability at various times relative to an IPSP, set up by a Ia quadriceps volley, which is shown at higher amplification and at the same sweep speed in I, and also plotted in J together with a tracing of the quadriceps volley (Q), as it entered the cord at the upper L 6 level. In K the reciprocals of the threshold current strengths (relative to the control strength), for the series partly shown in A–H, are plotted on the same time scale as for J, zero time being measured relative to the entry of the Q volley into the cord. The 0·2 and the 0·8 msec pulses are shown respectively as solid and hollow rectangles of appropriate length. In drawing the two inhibitory curves it is assumed that the pulses test the excitability at the mid point of their duration.

polarization by electrotonic spread. These observations have given further support to the suggestion discussed above: that under conditions of normal resting potential, inhibitory synaptic action produces virtually no IPSP, and that the IPSP recorded by an intracellular electrode owes its existence to membrane depolarization consequent on the electrode penetration.

When attempting to record inhibitory hyperpolarizations that have propagated electrotonically from motoneurones down the motor axons and so to the ventral root, it is important to realize that these potentials would be very small. On the average the IPSPs intracellularly recorded

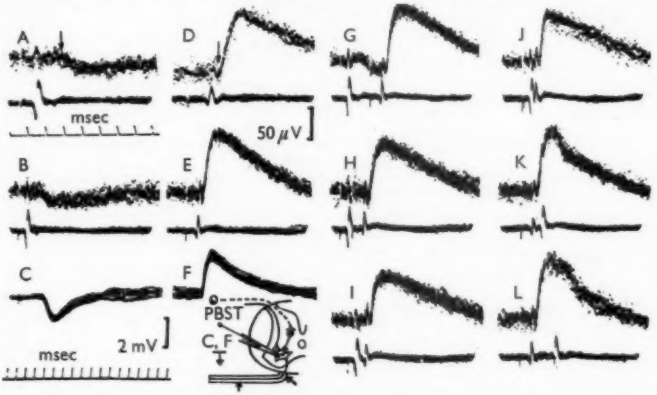

Fig. 8. C and F are intracellular records (see C, F in the inset diagram) of IPSPs and EPSPs evoked in a BST motoneurone at S 1 segmental level by a Ia quadriceps and a BST volley respectively, as shown in the inset diagram. The upper traces of the remainder are the potentials electrotonically conducted from the motoneurones along their motor axons and recorded from an isolated filament of the S 1 ventral root, one electrode being on the filament about 1 mm from its exit from the cord, the other at least 20 mm distally on the isolated filament, as shown by the two arrows in the inset diagram. A and B show potentials produced by a Ia quadriceps volley, D and E by a Ia BST volley. A and D are recorded at a faster time base, as shown, all other records being at same time base. G–L illustrate the interaction of EPSP and IPSP recorded in the ventral root when they are evoked at various volley intervals, as shown by the dorsal root records (from upper L 6) in the lower traces.

from PBST motoneurones in response to Q Ia volleys are several times smaller than the monosynaptic EPSPs generated by maximum Ia volleys in the PBST nerve. Furthermore, only a relatively small fraction of the fibres in any ventral root filament would be PBST motor axons, i.e. there would be a very effective shunting of any IPSP potential. Thus very high amplification must be employed and the potential change must be lifted out of the background noise by superposition of a great many superimposed traces. Figure 8 A and B shows that with such precautions a quadriceps

Ia afferent volley produced a well defined IPSP in a S1 ventral root filament about 1 mm after it emerged from the spinal cord. Monosynaptic EPSPs produced by a PBST volley were several times larger (D, E). Below B and E are shown on the same time scale the intracellularly recorded IPSP (C) and EPSP (F). The faster records, A and D, enable the latencies (note arrows) and rising phases of the ventral root IPSPs and EPSPs to be measured and compared. Comparison of B with C and E with

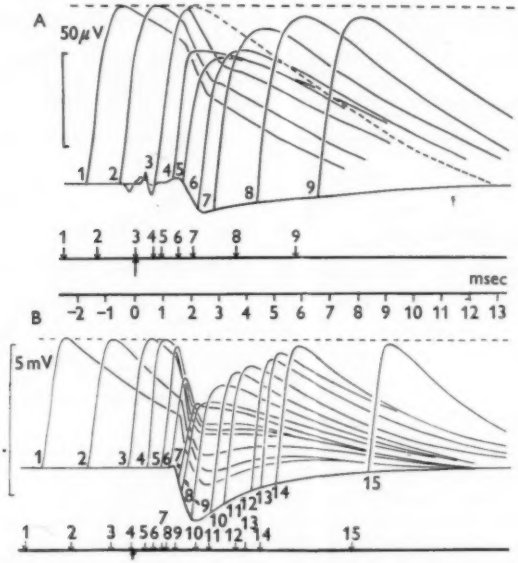

Fig. 9. Traces showing the interaction between IPSP and EPSP recorded electrotonically in S1 ventral root filament (A) and intracellularly from a motoneurone (B). Ia afferent fibres in PBST and quadriceps nerves are stimulated to give the excitatory and inhibitory volleys, respectively. Original records of A are in Fig. 8 G–L. Numbers at the foot of EPSPs are corresponding to those on the downward arrows which indicate the arrival time of PBST Ia afferent at upper S1 level. Upward arrows indicate the arrival of quadriceps volley at the same level. Same time scale for A and B.

F shows that latencies were virtually the same for the intracellular and for the ventral root IPSP and EPSP respectively; but, as would be expected, the summit was later and the decay much slower than with the respective intracellular potentials.

In G to L of Fig. 8 IPSP and EPSP were interacting at various volley intervals, B and E being the control records. Traces of these records in Fig. 9A show that the interaction closely paralleled the interaction ob-

served with intracellular recording (Coombs *et al.* 1955*b*; Curtis & Eccles, 1959). For example, in Fig. 9B there are plotted the intracellular potentials recorded from a motoneurone whose axon contributed to the potentials of Fig. 9A. In both types of recording it is seen that the IPSP was potentiated when the currents generating it were occurring during the depolarization of the EPSP. This potentiation occurred because the equilibrium potential for the IPSP was only at about 10 mV more hyperpolarization than the resting potential; hence a relatively small depolarization added considerably to the voltage driving the inhibitory ionic currents.

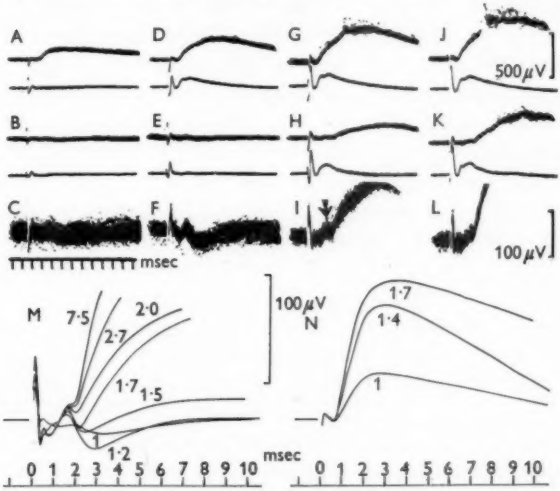

Fig. 10. Potentials recorded electrotonically on S3 ventral root. A, D, G and J; ipsilateral dorsal root stimulation. B, E, H and K; contralateral dorsal root stimulation. The stimulus is strengthened from the left to the right columns. In C, F, I and L the same potentials as in B, E, H and K, respectively, are shown with a higher amplification. Note the different voltage calibrations. Two sets of traces (M, N) below the photographs show the superposed potentials with different stimulus strengths, obtained by the contralateral dorsal root stimulation (M) and by the ipsilateral stimulation (N). Numbers in these figures indicate the strength of the dorsal root stimulation, relative to the weakest stimulus, which was just above threshold.

A hyperpolarizing potential was also recorded in the S3 ventral root in response to volleys set up by very weak stimulation of the contralateral S3 dorsal root. Such a potential was barely detectable with the low amplification of Fig. 10E, which seems comparable with that used by Lloyd & Wilson (1959), but was readily detectable at the higher amplification of Fig. 10F. The much smaller volley in Fig. 10B and C produced a very small hyperpolarization. These hyperpolarizations were much less

than the depolarizations produced by afferent volleys in the ipsilateral S3 dorsal root (Fig. 10A, D). When stronger stimulation was employed, the contralateral volley produced only a depolarization, in which, however, a small notch could be seen at the arrow in I, which presumably was produced by the onset of the now submerged inhibitory hyperpolarization. Not only was there a polysynaptic EPSP depolarization in H and I, but there was also an earlier small EPSP with a latency only a little longer than the ipsilateral monosynaptic EPSP in G, which presumably was due to the contralateral monosynaptic EPSP occasionally observed by Curtis *et al.* (1958) and by Frank & Sprague (1959). The superimposed traced records for contralateral S3 volleys (Fig. 10M) show the way in which the IPSP obtained with weakest volleys was submerged first by the contralateral monosynaptic EPSP and with stronger stimulation by the polysynaptic EPSPs. By contrast stronger ipsilateral stimulation merely added a later depolarization to the monosynaptic EPSP (Fig. 10N). Possibly the contralateral monosynaptic EPSP contributed to the failure of Lloyd & Wilson to detect the hyperpolarizing IPSP. It may be mentioned in this context that the monosynaptic EPSP produced by Q volleys in soleus motoneurones (Eccles, Eccles & Lundberg, 1957*b*) may submerge the Q IPSP propagated electrotonically along PBST motor axons, there apparently being a trace of this EPSP in Fig. 8A, B, before the start of the IPSP.

The largest IPSP recorded from filaments of the S1 or L7 ventral root was 17 μV, and the mean value was 10 μV in seven experiments with the Ia IPSP generated by Q volleys and 28 μV in five experiments with the contralateral S3 IPSP. The mean value for the Q IPSP was 11% of the monosynaptic EPSPs similarly recorded and evoked by PBST volleys, but it seems possible to reconcile this relatively small size with the mean values of the IPSPs and EPSPs determined by intracellular recording from a random sample of the motoneurones concerned. Thus in the 28 BST motoneurones investigated in the same experiments, the mean IPSP was 29% of the mean EPSP. The discrepancy (29% as against 11%) can at least be partly accounted for by the fact that PBST volleys generate an EPSP in many motoneurones, such as anterior biceps, which have no Q IPSP and which were rejected without being photographed. In addition, ventral root IPSPs in S1 and lower L7 root filaments are likely to be diminished by being superimposed on monosynaptic EPSPs which quadriceps Ia volleys generate in soleus motoneurones (Eccles *et al.* 1957*b*).

DISCUSSION

For the first time the central latency of Ia inhibitory action on monosynaptic reflexes has been directly measured, and has been found to be in excellent agreement with the latencies of the inhibitory post-synaptic

potentials, IPSP, recorded intracellularly from several motoneurones randomly sampled out of the population giving the reflex spike. The discrepancy between the latencies of the reflex inhibition on the one hand and of the IPSPs on the other was always less than 0·1 msec. In contradistinction, Lloyd & Wilson (1959) asserted that the central latency for inhibition of reflex discharge was as much as 1·0 msec shorter than the central latency for inhibition of the soma spike response of the motoneurone and that the IPSP would just be early enough to account for this soma inhibition. Thus there is a discrepancy of about 1·0 msec between our direct experimental measurements and the value calculated by Lloyd & Wilson from various indirect measurements.

It is not clear how Lloyd & Wilson arrived at this value of 1·0 msec. For example, in the construction of their Fig. 3 the spike *ghi* begins about 0·2 msec earlier than in the actual records from which it was derived and the spike summit, *h*, is drawn about 0·35 msec earlier than in their Fig. 1E. Possibly the time scale of Fig. 1 is incorrectly labelled. Be that as it may, superposition of their Fig. 1E and F gives a situation resembling the superimposed records of our Fig. 3 and reveals that the latency for the reflex spike inhibition was about 1·35 msec, which is in good agreement with the latencies observed in the present investigation when due allowance is made by subtracting from these values the conduction times in the motor axons.

The discussion of Renshaw's (1942) results on the central latency of reflex inhibition can also be criticized. For example, the latency was measured from a point at which there was no inhibition, rather than from a point derived from extrapolation of the inhibitory curve to the level of zero inhibition, which gives 0·95 msec for the latency of reflex inhibition as measured to the beginning of the reflex spike discharge from the motoneurones. The time to the summit of the reflex spike potential illustrated by Renshaw (0·35 msec) should be added to this figure, which gives 1·30 msec for the central latency of inhibitory action. When there has been a negligible longitudinal component in the inhibitory pathway, the IPSPs have often been observed with central latencies even briefer than 1·30 msec (Fig. 3J; Eccles *et al.* 1956; Curtis *et al.* 1958; Frank & Sprague, 1959), and consequently would just be in time to produce a reflex inhibition having the latency displayed by the reflex inhibition illustrated by Renshaw. On the contrary, Lloyd & Wilson (1959) derive a value of 1·15 msec for the inhibitory latency and then perform the irrelevant operation of subtracting therefrom the central latency for reflex discharge for motoneurones (0·95 msec), arguing that the remainder (0·2 msec) is inadequate for an interneuronal relay. The correct procedure would be to subtract from 1·15 msec the inhibitory synaptic delay of 0·3 msec and then enquire if the residual 0·85 msec is sufficiently long for conduction along a pathway to the motoneurone that traversed an interneuronal relay. Recently interneurones have been found in the intermediate nucleus that discharged an impulse in response to a Ia afferent volley which had entered the spinal cord only 0·5–0·8 msec earlier (Eccles, Eccles & Lundberg, 1960); hence the transmission time of 0·85 msec is possible with a disynaptic pathway, though our remeasured value from Renshaw's experiment would be 1·0 msec, and thus readily accommodated to a disynaptic pathway.

The diagram of Fig. 11 serves to illustrate the various factors that determine the central latency of Ia inhibitory action on motoneurones. The times of this diagram are all based on experimental measurements, and it will be seen that the shortest latency for inhibition of monosynaptic

reflex discharge could occur with an approximate simultaneity of the excitatory and inhibitory afferent volleys. The excitatory volley is shown producing an EPSP of the motoneurones after a latency of 0·5 msec, which is made up from 0·2 msec conduction time and 0·3 msec synaptic delay. (ESD). However, the EPSP takes a further 0·4 msec to rise to the threshold for the earliest spike discharge, and the time for reaching threshold

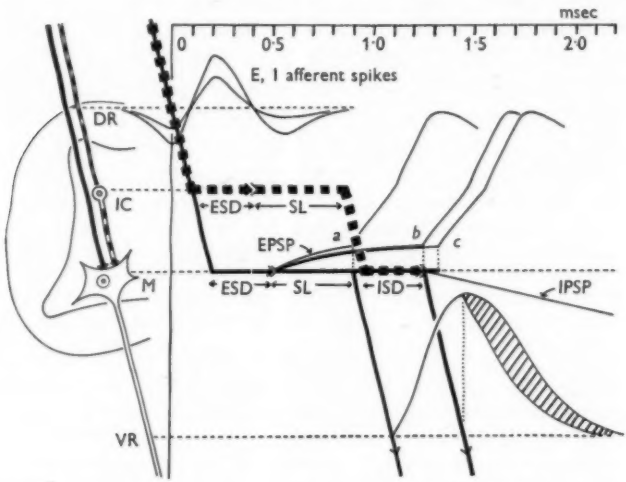

Fig. 11. Diagram showing time course of events during Ia inhibitory action on a monosynaptic reflex spike. The pathways are shown to the left with the monosynaptic excitatory line in black and the Ia inhibitory line in interrupted black. The remainder of the diagram is constructed both on the same time scale, as shown above, and on the spatial scale of the diagram to the left, except that the potential records are also shown in the conventional manner as rising from base lines at the respective recording sites: dorsal root entry, DR; the motoneurone, M; and the ventral root, VR. The slopes of the lines on the spatial–temporal co-ordinates give the velocities, and delays at regions of junctional transmission are given by lengths of the horizontals there. Thus the monosynaptic path (the E afferent spike at DR) is shown delayed at the motoneurone by an excitatory synaptic delay ESD of 0·3 msec in initiating the EPSP, and a spike latency, SL, of 0·4 msec for the shortest latency (*a*) and 0·75 msec for the longest (*c*) involved in the EPSP initiating a spike discharge. The spikes discharges by *a* and *b* are shown propagating into the ventral root. An I afferent spike arriving synchronously with E, as shown by records at DR, and having an equivalent length of central pathway, is shown delayed for 0·75 msec at the intermediate cell (IC) relay (0·3 msec of excitatory synaptic delay plus 0·45 msec of spike latency); and thereafter propagating to the motoneurone, having a further delay of 0·3 msec (the inhibitory synaptic delay, ISD) before initiating the IPSP after a total central latency of 1·25 msec. It will be seen that this IPSP is just produced in time to delay or suppress (cf. Fig. 1K) all reflex discharges after *b* (for example *c*), and so is just able to diminish the height of the reflex spike as shown by the hatched area.

is as long as 0·8 msec with the latest discharges that contribute to the summit of the reflex spike potential in the ventral root, as is shown in the potential–time diagrams in this figure. Meanwhile the inhibitory volley will have entered the intermediate nucleus (IC), and, after a synaptic delay of 0·3 msec (ESD), will have produced an EPSP in the Ia intermediate cells, which rises to the threshold for generating the discharge of impulses after a further 0·45 msec, values which conform with experimental values for the quickest responses of intermediate neurones activated by Ia impulses (Eccles *et al.* 1956; Curtis *et al.* 1958; Eccles *et al.* 1960). Within about 0·4 msec (0·3 msec for the inhibitory synaptic delay, ISD) the impulses of the intermediate cells will be initiating an IPSP of the motoneurones that has a central latency of 1·25 msec and which is just in time to inhibit the discharge of the latest motoneuronal discharges contributing to the reflex spike potential. It will be evident from Fig. 11 that the interval between the inhibitory and excitatory volleys provides a most unsatisfactory basis from which to derive the central latency for inhibitory action, and this criterion has seriously misled Lloyd and Wilson.

There is satisfactory agreement between the time courses for Ia inhibition of monosynaptic reflexes (Figs. 4B, 5G) and the IPSPs that are recorded both from the ventral root and from motoneurones (Figs. 4A, 5F, 7I, J, 8A–F, 10C, F). The low residuum of inhibitory action persisting for more than 10 msec (Fig. 4B) would be expected if inhibitory synaptic action on motoneurones produced a hyperpolarizing potential that continued the depression of excitability beyond the brief phase of high ionic permeability (Curtis & Eccles, 1959). Thus it can be concluded that the IPSP recorded by an intracellular electrode is not simply a consequence of a depolarization produced by impalement with a micro-electrode, as suggested by Lloyd & Wilson (1959) and Jack *et al.* (1959).

In any case, it would not be expected on theoretical grounds that a prolonged steady membrane depolarization would provide conditions giving a hyperpolarizing inhibitory potential if there were no inhibitory potential at the resting membrane potential. There is independent evidence that the equilibrium potential for potassium, E_K, is at a more hyperpolarized level than the resting membrane potential, for the after-hyperpolarization that follows a spike response of the motoneuronal membrane is due to a raised K^+ permeability (Coombs, Eccles & Fatt, 1955*a*; Eccles, 1957); and it would be generally agreed that this after-hyporpolarization occurs in motoneurones independently of any lowering of membrane potential by injury, for it can readily be observed after electrotonic propagation to the ventral root (Eccles & Pritchard, 1937; Gasser, 1939; Lloyd, 1951). Thus the equilibrium potential for the IPSP would be identical with the resting membrane potential, E_R, only if the movement of K^+ ions made no contribution to the inhibitory post-synaptic action. Cl^- ions would therefore be almost exclusively concerned in the high ionic permeability occurring in the inhibitory response of the post-synaptic membrane, which would be in agreement with the general conclusion that with nerve and muscle the equilibrium potential for Cl^- ions (E_{Cl}) is identical with the normal resting membrane potential E_R (Conway, 1957; Hodgkin, 1958; Hodgkin & Horowicz, 1959; Keynes, 1954). If now E_R is lowered to

$E_{R'}$ by micro-electrode penetration, E_{Cl} would be at a more hyperpolarized level than $E_{R'}$, and inhibitory synaptic action would cause a hyperpolarizing response, even if none was present normally. But the difference between E_{Cl} and $E_{R'}$ would cause also a net influx of Cl ions until the redistribution caused the new equilibrium potential, $E_{Cl'}$, to be identical with $E_{R'}$ (cf. Boistel & Fatt, 1958). The time course of this distribution is quite rapid, having a half-time of about 15 sec (Coombs *et al.* 1955*b*; Eccles, 1957; J. C. Eccles, R. M. Eccles & M. Ito, unpublished observations), so a steady membrane depolarization resulting from electrode penetration would not account for IPSPs that persisted for more than 1–2 min; and hyperpolarizing IPSPs have been regularly observed during long periods of steady membrane potential.

A rectangular pulse of depolarizing current will generate the discharge of an impulse from a motoneurone when it has depolarized the initial segment of the motoneurone to a critical level (Araki & Otani, 1955; Frank & Fuortes, 1956; Coombs *et al.* 1957*a*, *b*). In doing this the applied current will pass partly through the membrane capacity, removing its charge so that the critical level is attained, and partly through the membrane conductance. When the stimulating current is as brief as 0·2 msec, it has to be very intense in order to depolarize the membrane to the critical level for impulse generation; and it would not be appreciably antagonized by the flow of inhibitory current. The inhibitory curve would be expected to be virtually identical with the time course of membrane hyperpolarization (the IPSP), as is actually seen in Fig. 7K (short rectangles). On the other hand, a stimulating current of 0·8 msec duration would be of much lower intensity (only 30% in Fig. 7), as may be seen by reference to the strength–latency curves for motoneurones (Frank & Fuortes, 1956; Coombs *et al.* 1959), and consequently it would be very effectively antagonized by the inhibitory current as well as by the hyperpolarization; hence the dual composition of such inhibitory curves (Fig. 7K, long rectangles). The excitatory synaptic currents generating the monosynaptic EPSP and spike discharge have an effective duration even in excess of 0·8 msec (Curtis & Eccles, 1959; C. Terzuolo & T. Araki, personal communication), so inhibitory current flow would tend to have the dominating influence in preventing the generation of spikes, the hyperpolarization being relatively unimportant, as is illustrated in the inhibitory curves of Figs. 4, 5.

The relative sizes of the two phases of inhibitory action could be quantitatively evaluated if the electric time constant of the membrane were known. Unfortunately there is a large discrepancy between the time constant calculated from strength–latency curves, which has a mean value of 1·1 msec (Frank & Fuortes, 1956; Coombs *et al.* 1959) and that derived from the time course of membrane potential change produced by a rectangular pulse (mean value 3·1 msec, Coombs *et al.* 1959). Presumably the membrane potential changes produced by depolarizing current pulses would be related to the former value of 1·1 msec rather than to the longer

value, and thus the dominance of the initial brief phase in Figs. 4, 5, 7K (long rectangles) is to be expected.

Wilson & Lloyd's statement that the 'latency for inhibition of soma spike is no measure of the latency of reflex inhibition' is a confusing half-truth. It is generally accepted that the spike recorded by a micro-electrode in the soma of a motoneurone is compounded of an initial IS (or A) spike and a later SD (or B) spike (Araki & Otani, 1955; Fatt, 1957; Fuortes *et al.* 1957; Coombs *et al.* 1957*a*, *b*) and that the former signals the generation of an impulse by the motoneurone and is responsible for the discharge of impulses along the motor axon, whereas the SD spike (often called soma spike) arises later by a secondary invasion. Thus, in the above statement, Wilson & Lloyd would be correct with this usage of 'soma spike' as the SD spike; their confusion arises because they use the same phrase 'soma spike' in two senses in the same argument, i.e. for all spikes recorded in the soma (IS plus SD) and for SD spike alone.

CONCLUSIONS

In contradistinction to Lloyd & Wilson (1959) it can be concluded:

(*a*) That the time course of IPSP gives a complete explanation of the observed inhibition of impulse discharge from motoneurones, both that arising reflexly and that in response to direct electrical stimulation.

(*b*) That there is a latency differential of about 0·8 msec between the Ia IPSP and the monosynaptic EPSP, which conforms with the requirements of a disynaptic inhibitory pathway.

(*c*) That inhibitory synaptic action on a motoneurone results from two processes: a brief phase of high ionic conductance of the inhibitory subsynaptic membrane; and a consequent hyperpolarization, the IPSP, which occurs even at the normal resting membrane potential.

(*d*) That the spike potential recorded in the soma of motoneurones provides a reliable indicator of the generation of impulse discharge from motoneurones, both under normal conditions and when under inhibitory action.

SUMMARY

1. The central latency of inhibition has been measured by observing the onset of its action upon the rising phase of a suitably timed monosynaptic reflex spike recorded in the ventral root.

2. With the inhibition of biceps-semitendinosus motoneurones by quadriceps Ia volleys and with the contralateral inhibition of motoneurones at sacral 3 level, the latency of inhibitory action on the reflex discharge of impulses from motoneurones into the ventral root has been found always to be within 0·1 msec of the intracellularly recorded inhibitory post-synaptic potentials (IPSPs).

3. In a reinvestigation it was confirmed that the time course of inhibitory action on monosynaptic reflexes by a Ia inhibitory volley had a brief intense phase and a longer low-intensity tail that persisted for about 10 msec.

4. A similar time course was also observed when the effect of the inhibitory volley was tested by the raised threshold to relatively long depolarizing current pulses (up to 1 msec) applied through the intracellular electrode. With testing by much briefer pulses the raised threshold had a time course resembling the IPSP.

5. IPSPs have been regularly recorded from ventral roots after electrotonic transmission from motoneurones.

6. These experimental results have been discussed in detail and it has been concluded that the time course of the IPSP gives a complete explanation of the observed inhibition of reflex discharge from motoneurones, the inhibitory post-synaptic currents being responsible for the initial brief intense phase that is superimposed upon an inhibitory effect attributable to the hyperpolarization of the IPSP.

7. It is shown that the central latency of inhibitory action exactly corresponds with the latency required for a pathway with an interpolated interneurone.

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HYALURONIDASE AND ANTIDIURETIC ACTIVITY IN URINE OF MAN

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Until recently the action of the antidiuretic hormone has been described as restoring 'water balance by promoting the reabsorption of the osmotically free water left by the distal reabsorption of Na. Under the action of the hormone this water is reabsorbed; in its absence this water is excreted' (Smith, 1956). Wirz (1956) has suggested an explanation of the mechanism of water reabsorption. He, and later Gottschalk & Mylle (1959), showed by micropuncture study in the rat that fluid in the first half of the distal tubule was hypotonic to plasma under all conditions of hydration, but that in dehydrated animals it became isotonic in the second half of the distal tubule and hypertonic in the collecting ducts. In the kidney producing a concentrated urine the tubular fluid comes into osmotic equilibrium first with the cortical tissue and later, in the collecting tubules, with the medullary tissue which is known to be increasingly hypertonic towards the papilla. According to Wirz's theory, the essential action of vasopressin is to increase the permeability to water of the distal parts of the nephron and collecting ducts. In 1958 Ginetzinsky found that the urine of several mammals contained hyaluronidase: this disappeared during water diuresis, but was present during osmotic diuresis, in the dog. In a histological study in rats Ginetzinsky observed that the cement substance between the cells of the collecting tubules reacted as hyaluronic acid when the animals were water-loaded, but as its polymerization products when they were dehydrated. He concluded that when stimulated by the antidiuretic hormone, the cells of the collecting tubules secrete hyaluronidase, which in turn depolymerizes the mucopolysaccharide complex of the basement membrane of the tubules, hence making 'the structures separating the tubule lumen from the interstitial tissue permeable to water. The hypotonic fluid in the tubules then follows the osmotic gradient and undergoes facultative reabsorption' (Ginetzinsky, 1958). This was a new approach to a process which had hitherto been difficult to envisage. It seemed, therefore, of interest to test Ginetzinsky's hypothesis by comparing the excretion of hyaluronidase and antidiuretic activity in urine under various conditions in man.

METHODS

The human subjects were eight normal adults (four men and four women), eight new-born male infants, one patient suffering from diabetes insipidus and two children with an inborn renal disease known as 'nephrogenic diabetes insipidus'. The adult patient with diabetes insipidus was a man of 27 years, whose condition was controlled by intramuscular injections of 10 u. Pitressin tannate administered every other day. When seen, the treatment having been interrupted for 48 hr, the patient was excreting urine at an average rate of 14 ml./min. The children with 'nephrogenic diabetes insipidus' were two cousins, aged 4 and 17 respectively. Both had been treated with chlorothiazide. 'Nephrogenic diabetes insipidus' is a hereditary disease transmitted through the mother in the same way as haemophilia, and characterized by an inability of the kidneys to respond to vasopressin, and hence to concentrate urine, though the post-pituitary gland appears to be unaffected.

Diuresis, when required, was induced by the ingestion of water in varying amounts or of ethanol (35–45 ml. in 200 ml. water). Antidiuresis in hydrated subjects was obtained by intramuscular injections of 500 m-u. vasopressin. In all cases urine was collected without catheterization. In new-born infants the sample of urine collected was that which was first voided after birth, before the first feed and thus at a time when the baby was dehydrated.

Estimation of hyaluronidase in urine. Hyaluronidase was determined by the change in viscosity induced when incubated with hyaluronic acid by the method of McClean & Hale (1941) with certain modifications. In order to minimize the action of interfering substances such as ascorbic acid and to concentrate the hyaluronidase, the urine samples were first taken nearly to dryness by exposure to dialysis tubing containing polyethylene glycol, as described by Kohn (1959), and reconstituted with water to represent a urine flow of 0·1–0·15 ml./min. Since the original rate of urine flow was unknown in the new-born infants, the samples were concentrated three- to eightfold, according to the volume available.

0·2 ml. hyaluronic acid + 0·05 ml. reconstituted urine + 0·05 ml. citrate buffer pH 4·6 (containing 8 % NaCl) was incubated at 34° C for 90 min and the viscosity then determined at room temperature. The hyaluronidase concentration was obtained from a curve derived from different amounts of the standard hyaluronidase preparation (Light and Co; 500 u./mg) under the same conditions. Boiled urine produced little or no change in the viscosity of the hyaluronic acid. The latter was a preparation of mixed mucopolysaccharides from human umbilical cord and was not of uniform viscosity. The solution used was of such a concentration that, diluted as indicated above, it took approximately twice as long (110 sec) in the viscosimeter as water (55 sec). The time was reduced to 101, 91 and 79 sec by 0·5, 1·0 and 2·0 u. hyaluronidase respectively. A difference of < 5 sec between the unknown and hyaluronic acid was not considered quantitatively significant. The absolute values varied with day-to-day variations in room temperature, but this was steady to $\pm 0{\cdot}5°$ C during any one set of readings.

Identification of the viscosity-reducing material in urine as an enzyme, apart from its destruction by boiling, was provided by a typical curve of activity versus time. Its action at 30, 60 and 90 min incubation was 33, 55 and 68 % of that at 3·5 hr, in comparison with 59, 72 and 79 % for the standard preparation (bull testis). Its identity as a hyaluronidase was further substantiated by inhibition of its viscosity-reducing action by Suramin to the same degree as that of the standard preparation.

Estimation of antidiuretic activity in urine. The method was that described by Dicker (1953), using rats under ethanol anaesthesia and kept with a constant water load of 8·0 ml./100g body weight. The estimations were done as a (2 + 2) doses assay, with a commercial preparation of vasopressin as standard, and the antidiuretic activity expressed in terms of the standard used. By a suitable modification in the timing of hydration (Thorn, 1957), the sensitivity of test animals was increased, with the result that the smallest amount of antidiuretic activity assayable was that corresponding to 0·025 m-u. vasopressin/ml. Urine samples containing antidiuretic activity were treated with sodium thioglycollate. In all

but one case (subject Wh. Table 1) this resulted in the complete inactivation of the antidiuretic activity, suggesting that the latter was of neurohypophysial origin.

The commercial preparation of vasopressin used for injections as well as for assays was Pitressin (Parke, Davis and Co; batch LZ 231 A): it contained lysine vasopressin only, as shown by paper chromatography. It was labelled as containing 20 u. pressor activity/ml.: in this paper in stating both doses and results of assays 1 u. means 0·05 ml. of the preparation.

RESULTS

Water and alcohol diuresis. The results of eight experiments on adult subjects are given in Table 1. In all experiments the concentration of both antidiuretic activity and hyaluronidase fell dramatically at the height of the diuresis and increased again as the urine flow returned to its resting value, 40–90 min later. The absolute excretion rate of both hyaluronidase and antidiuretic activity followed the same course in all experiments. There was, however, a greater variation in the excretion of the antidiuretic activity at similar rates of urine flow (0·12–10·0 m-u./min), not only in different individuals but in the same individual on different occasions. Variation occurred also in hyaluronidase excretion but to a much less degree (0·52–4·42 u./min).

TABLE 1. Excretion of antidiuretic activity and hyaluronidase before, during and after water and alcohol diuresis

Subject	Urine flow (ml./min)	Antidiuretic activity (m-u./ml.)	Antidiuretic activity (m-u./min)	Hyaluronidase (u./ml.)	Hyaluronidase (u./min)
		Water diuresis			
Wh.	0·38*	10·64	4·04	0·90	0·34
	14·80*	0·53	7·80	0·00	0·00
	1·23*	8·10	10·00	1·20	1·48
Dic.	3·00	1·82	5·46	0·85	2·55
	9·00	0·00	0·00	0·28	2·52
	1·00	2·40	2·40	3·85	3·85
Im.	0·86	4·75	4·10	2·20	1·90
	6·13	0·00	0·00	0·00	0·00
Eg.	0·68	3·35	2·28	4·60	3·13
	5·60	0·00	0·00	0·14	0·78
	0·63	5·17	3·25	2·00	1·26
		Alcohol diuresis			
Gi.	0·90	0·12	0·11	1·32	1·19
	11·50	0·00	0·00	0·00	0·00
	1·50	0·11	0·16	1·15	1·73
Di.	9·00	0·00	0·00	0·00	0·00
	1·00	0·12	0·12	3·05	3·05
Dia.	1·16	1·60	1·86	3·80	4·42
	7·70	0·37	2·85	0·00	0·00
	1·14	1·25	1·43	1·50	1·71
Eg.	0·53	0·10	0·53	5·00	2·65
	0·61	0·00	0·00	2·20	1·32
	0·44	0·11	0·05	7·80	3·44

* Sodium thioglycollate destroyed about 45% only of the antidiuretic activity of these urine samples.

Injection of vasopressin. Injection of vasopressin into hydrated subjects is known to reduce the rate of urine flow, and it was of obvious interest to see whether this was accompanied by an increase in excretion of hyaluronidase. An intramuscular injection of 500 m-u. vasopressin was made, therefore, into three normal subjects and one patient with diabetes insipidus. The results given in Table 2 show that hyaluronidase concentration in the urine and its total output were considerably increased in all four subjects during 65–90 min following the injection. The antidiuretic activity excreted in the urine during this period amounted to 4½–11 % of that of the injected material.

TABLE 2. Excretion of hyaluronidase and antidiuretic activity following an intramuscular injection of vasopressin into normal subjects

Subject	Time after injection (min)	Urine flow (ml./min)	Hyaluronidase excretion		Antidiuretic activity	
			(u./ml.)	(u./min)	(m-u./ml.)	Total (m-u.)
Mrs B.	—	2·08	0·41	0·85	0·00	—
	Vasopressin 500 m-u. intramuscularly					
	0–55	0·62	2·25	1·40	0·81	27·6
	55–70	1·33	1·40	1·86	0·76	15·2
E.	—	3·95	0·36	1·42	0·00	—
	Vasopressin 500 m-u. intramuscularly					
	7–30	1·28	1·80	2·30	0·11	3·4
	30–50	0·59	5·50	3·25	0·64	8·0
	51–90	0·68	3·23	2·20	0·44	11·9
Mrs C.	—	1·24	0·84	1·04	0·00	—
	Vasopressin 500 m-u. intramuscularly					
	0–45	1·51	1·54	2·33	0·30	20·1
	45–85	0·93	2·74	2·55	0·98	36·3
H.H. (diabetes insipidus)	—	14·00	0·00	—	0·00	—
	Vasopressin 500 m-u. intramuscularly					
	0–30	2·30	1·40	3·22	0·31	21·5
	30–75	3·45	0·45	1·55	0·14	21·7

The results of an intramuscular injection of vasopressin in two cases of nephrogenic diabetes insipidus (Table 3) stand in sharp contrast with those given in Table 2. The rate of urine flow was actually increased after the injection of vasopressin, possibly owing to an increase in filtration rate, as suggested by an increase in creatinine excretion from 14 to 16·5 mg/hr. There was no hyaluronidase in the urine, either before or after the injection of 500 m-u. vasopressin but a far higher proportion of the injected antidiuretic activity was excreted: 47 and 80 % in 65–75 min as compared with 5–15 % in normal individuals.

New-born infants. In eight new-born infants appreciable amounts of antidiuretic activity were found in the urine, accompanied by hyaluronidase.

The results given in Table 4 show only concentrations, as the estimations were made on the first sample of urine voided after birth, and rates of flow were therefore unknown. Though the concentration of antidiuretic activity is relatively low in comparison with that of resting urine flow in most of the adults, that of hyaluronidase falls within the adult range. As in the adults, there is a wide variation in the ratio of antidiuretic activity to hyaluronidase concentration.

TABLE 3. Excretion of hyaluronidase and antidiuretic activity following the injection of vasopressin in cases of nephrogenic diabetes insipidus

Subject	Time after injection (min)	Urine flow (ml./min)	Hyaluronidase (u./ml.)	Antidiuretic activity (m-u./ml.)	Antidiuretic activity Total (m-u.)
B.B. 4 yr	—	1·26	0·00	0·00	—
	—	2·93	0·00	0·00	—
	Vasopressin 500 m-u. intramuscularly				
	0–45	4·04	0·00	1·18	215
	45–75	3·75	0·00	1·62	182
D.C. 17¼ yr	—	3·33	—	—	—
	Vasopressin 500 m-u. intramuscularly				
	0–82	3·93	0·00	0·73	235
	82–130	3·37	0·00	0·48	78

TABLE 4. Excretion of antidiuretic activity and hyaluronidase in new-born babies

Subject	Antidiuretic activity m-u./ml.	Hyaluronidase u./ml.
Bot.	0·025	4·0
H.	0·025	0·7
Bog.	0·056	3·3
Ba.	0·074	1·7
F.	0·150	5·2
Mu.	0·237	5·3
S.	0·875	5·0
Ma.	0·900	4·0

DISCUSSION

The results presented suggest that there is some relation between antidiuretic activity in the urine and hyaluronidase liberation in the kidney, as suggested by Ginetzinsky (1958), and are in apparent conflict with the negative findings on man of Berlyne (1960), who found no relationship between hyaluronidase excretion and rate of urine flow. Berlyne, however, gives little detail, and presumably only one analysis was made on each individual in water, mannitol or potassium diuresis. Of his water-diuresis subjects, only one had a urine flow greater than 2 ml./min. This, in conjunction with the wide individual variation in hyaluronidase excretion both in resting urine and in response to ingestion of water, as seen in Table 1, would appear to offer sufficient explanation of his apparently negative results.

The increase in hyaluronidase excretion following intramuscular injection of vasopressin, both in normal subjects and in a case of diabetes insipidus, strengthens the suggestion that hyaluronidase is concerned in the production of a concentrated small volume of urine. The lack of any increase following a similar injection in two cases of nephrogenic diabetes insipidus further suggests that vasopressin can have no antidiuretic action other than by liberating hyaluronidase and affords strong support to Ginetzinsky's conclusion that the action of vasopressin is to render more permeable to water the collecting tubules in their hypertonic surroundings. The genetically-controlled missing link is clearly somewhere in the vasopressin–hyaluronidase probable chain of reactions, but cannot at present be more accurately located.

The failure of new-born infants to produce as concentrated a urine as adults cannot be ascribed to such an inability. They excrete both antidiuretic activity and hyaluronidase in appreciable concentration. Heller (1944) found that, whereas in infants a few days old an injection of posterior pituitary extract caused some reduction in urine flow with increase in its concentration, two new-born infants so tested gave no response. Their urine was more concentrated than that of the older infants and their kidneys were presumably responding maximally to endogenously produced vasopressin. Heller's results taken in conjunction with our own suggest that vasopressin and its tubular action necessary for the production of concentrated urine in the adult are already present in the new-born baby. The most likely cause of failure of the new-born infant to produce a concentrated urine is failure to develop hypertonicity of the renal medulla which surrounds the collecting ducts in the adult. The kidney of the new-born child is known to be immature in many respects and it would not be surprising if the loop of Henle 'counter-current' system is not fully developed.

The lack of any regular quantitative relation between the excretion rates of antidiuretic activity and hyaluronidase requires some explanation. The antidiuretic activity appearing in the urine is presumably related to vasopressin as in all but one case (see Table 1) it was inactivated by sodium thioglycollate; it appears to be filtered at the glomerulus, whereas hyaluronidase probably spills over from collecting-tubule cells, as suggested by Ginetzinsky on histological grounds. In the normal subject only some 10–15 % of injected vasopressin is recovered from the urine, whereas in the case of nephrogenic diabetes insipidus up to 80 % was excreted in the urine during 65 min following the injection. It is tempting to postulate that vasopressin actually gets destroyed during its stimulation of hyaluronidase liberation from the tubule cells. In such a complex situation, it would be unreasonable to expect any quantitative relation between the excretion rates of the two substances.

SUMMARY

1. Following the ingestion of water or alcohol, antidiuretic activity and hyaluronidase concentrations in the urine fell at the height of diuresis and increased again with restoration of normal rate of urine flow. The total excretion of hyaluronidase followed the same pattern.

2. Injection of vasopressin in two cases of nephrogenic diabetes insipidus caused no excretion of hyaluronidase, in contrast with three normal subjects and a case of diabetes insipidus, in whom a large excretion of the enzyme occurred.

3. The nephrogenic diabetes insipidus patients excreted 50–80% of the injected antidiuretic activity in contrast with the normal 5–15%.

4. The urines of eight new-born babies contained appreciable concentrations of both antidiuretic activity and hyaluronidase.

5. It is concluded that the antidiuretic hormone acts on the kidney by liberating hyaluronidase and that the resultant decrease in rate of urine flow is probably due to increased permeability of the collecting tubules, allowing the concentration of their contents to come into osmotic equilibrium with their hypertonic surroundings.

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THE SODIUM–POTASSIUM HYPOTHESIS AS THE BASIS OF ELECTRICAL ACTIVITY IN FROG VENTRICLE

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The aim of this study was to see whether the action potential of cardiac muscle can be explained in terms of a sodium–potassium theory similar to that proposed by Hodgkin & Huxley (1952). There is much evidence supporting the idea that an exchange of sodium and potassium ions underlies the electrical activity of heart muscle. Thus, conduction in cardiac tissue is blocked in sodium-free solutions (Overton, 1902; Clark, 1913; Draper & Weidmann, 1951; Brady & Woodbury, 1957). It is generally agreed that the membrane potentials recorded intracellularly in all cardiac tissue except pace-maker tissue reverse during depolarization (Cranefield, Eyster & Gilson, 1951; Draper & Weidmann, 1951, Brady & Woodbury, 1957). Weidmann (1951, 1955) has demonstrated an increase in the membrane slope conductance in Purkinje tissue during depolarization and a dependence of the maximum depolarization rate on the external sodium concentration and the initial resting potential.

The data on exchange of radioactive ions in heart muscle are difficult to interpret. However, Johnson's (1957) results suggest that the exchange of sodium ions in frog ventricle is sufficient to account for depolarization. Similarly, Wilde, O'Brien & Bay (1956) found a pulsatile increase in potassium efflux from beating turtle ventricle at about the time of rapid repolarization. The membrane resting potentials of cat auricle (Burgen & Terroux, 1953) and of the Purkinje fibres in calf and sheep (Weidmann, 1956*a*) depend upon the external potassium concentration. On the other hand, Coraboeuf & Otsuka (1956) found that the peak height of the action potential of guinea-pig ventricle does not behave like a sodium electrode, but that conduction is blocked in a sodium-free medium after 15–20 min. Although apparently contradictory, this observation can be explained within the framework of the sodium–potassium theory (see Discussion).

The experiments described here support the view that the sodium–potassium theory, suitably modified, adequately accounts for the electrical

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activity of frog ventricle. This evidence comes from studies of changes in the membrane resting and action potentials produced by alterations in the concentrations of sodium and potassium in the perfusate and by changes in the excitation rate.

METHODS

Isolated, cannulated ventricles of *Rana pipiens* were used throughout. Lack of coronary circulation, ease of cannulation of the ventricle and a maximum diffusion distance of 200 μ to any fibre are the major advantages of this experimental preparation.

Perfusion

After the hearts were excised they were perfused by way of a cannula tied into the ventricle through the atrioventricular valve. Outflow usually took place through a cannula tied into the truncus arteriosus. Solutions of different ionic composition were led into the perfusion chamber in separate lines to a common junction near the input cannula. Part of the flow by-passed the cannula to perfuse the exterior of the ventricle. The total dead space, exclusive of the perfusion chamber but including the ventricular chamber, was approximately 0·3 ml. The half-time for change of solution was 10 sec, estimated from the rate at which the duration of the action potential changed in a solution in which the concentration of Na^+ was half normal. The composition of the perfusates was (mM): NaCl, 110; KCl, 2·5; $CaCl_2$, 1·08; $NaHCO_3$, 2·38; K_2HPO_4, 0·08; glucose, 5·5. When the concentration of Na^+ was altered, osmolarity was maintained with either choline chloride or sucrose. No osmotic compensation was made for small changes in the concentration of K^+. Solutions with high concentrations of K^+ (above 10 mM) were obtained by diluting isotonic KCl with normal Ringer's solution. The temperature of the perfusates was 18° C unless otherwise noted.

Stimulus

The stimulus was applied between a large sheet of platinum (20 cm^2) coiled in a side arm of the input cannula, and a platinum electrode lining the perfusion chamber. A 15 mA rectangular stimulus pulse, 2 msec in duration and floating with respect to earth, was applied through a 0·1 μF condenser to reduce polarization of the electrodes. A small coil of platinum wire earthed the solution in the chamber at a point selected to give minimum stimulus artifact in the recording system. Some polarization of the stimulus electrodes was evident and necessitated a small correction to the overshoot of the action potential. The correction was obtained from the difference between the recorded action potential and the stimulus artifact recorded just outside a fibre. In order to avoid shunting of the stimulus current, the flow of the solution by-passing the cannula was regulated so that it fell in drops into a side arm of the chamber containing the heart.

Intracellular potentials

Intracellular recording was accomplished with flexibly mounted 0·5 μ Ling–Gerard microelectrodes (Woodbury & Brady, 1956). Momentarily returning the 10^{10} Ω grid resistor of the input circuit to earth through a 10 V source gave a direct measurement of electrode resistance. In this case the current through the grid resistor was such that the voltage change at the input in millivolts was equal to the electrode resistance in megohms. Electrode resistances ranged from 30 to 100 MΩ. Tip potentials (Adrian, 1956) were not measured and may be partly responsible for the large observed variation in resting potentials. Voltage calibrations were obtained by applying −10, −50 and −100 mV potentials across a small resistance in the indifferent lead from the perfusion chamber to earth.

An RC circuit with an appropriate time constant at the output of the voltage amplifier performed the differentiating operation. The time constants used were: 5 msec for phases

1 and 2, 1 msec for phase 3 and 0·05 msec for depolarization. The derivatives were calibrated by inserting a 150 mV sawtooth wave which had a slope of 0·10–1·0 V/sec between earth and the recording chamber.

DEFINITIONS AND APPROXIMATIONS

Definiton of symbols

$[Na^+]_o$, $[Na^+]_i$, ...	Ion concentrations in external and internal solutions (mM).
V	Membrane potential taken as potential of inside minus potential of outside (mV).
V_{Na}, V_K, V_{Cl}	Equilibrium potentials of Na^+, K^+ and Cl^-; e.g. $V_{Na} = 58 \log_{10}([Na^+]_o/[Na^+]_i)$ mV.
$\dot{V}$	Rate of change of V with respect to time (V/sec).
$\dot{V}_d$, $\dot{V}_1$, $\dot{V}_2$, $\dot{V}_3$	V at the four successive inflexion points of the action potential.
I_m	Membrane current density; from definition of V, outward current is positive (A/cm²).
I_i	Membrane ionic current density (A/cm²).
I_{Na}, I_K, I_{Cl}	Respective ionic currents (A/cm²).
C	Membrane capacity (μF/cm²).
a	Mean fibre radius, taken as 5 μ.
ρ_i	Specific resistivity of myoplasm, taken as 200 ohm.cm.
u	Conduction velocity, taken as 20 cm/sec.
t	Time (sec or msec).
t_{AP}	Duration of action potential measured between points of 10 mV depolarization.
t_s	Time interval between successive stimuli.
τ	Resting membrane time constant.
T_s, T_l	Short and long time constants of g_{Na} inactivation.
AP	Action potential.
g, g_{Na}, g_K, g_{Cl}	Total, Na^+, K^+, Cl^- membrane chord conductances (mmho/cm²).
G, G_{Na}, G_K, G_{Cl}	Total, Na^+, K^+, Cl^- membrane slope conductances (mmho/cm²).
r, a, p	Subscripts denoting values of any variable at rest, at the peak of activity, and in the plateau period respectively.

Approximations

The total membrane current flow is the sum of the ionic and capacitive components:

$$I_m = I_i + C\dot{V}. \tag{1}$$

If local (i.e. total membrane) current flow is negligible compared with ionic flow, then the ionic current through the membrane at any time is proportional to the time derivative of the action potential:

$$I_i = -C\dot{V}. \tag{2}$$

Local current flow is proportional to the second spatial derivative of V. If activity propagates at constant velocity, then local flow is also proportional to $\ddot{V}$, the second time derivative of V, i.e. $I_m = a\ddot{V}/2\rho_i u^2$.

The validity of the approximation in equation (2) can be checked by estimating membrane current when V is maximum on the rising and falling phases of the action potential and by comparing these estimations of the current with the corresponding values of $C\dot{V}$. Representative values of $\ddot{V}$ and $\dot{V}$ are 2×10^4 V/sec² and 10 V/sec for the rising phase, and 80 V/sec² and 0·4 V/sec for the falling phase. If $C = 30$ μF/cm² (Trautwein, Kuffler & Edwards, 1956), the calculated peak local current is 6×10^{-5} A/cm² during depolarization, while $C\dot{V} = 30 \times 10^{-3}$ A/cm². Thus, when $\ddot{V}$ is maximum, I_m is only 20 % of $C\dot{V}$, so $-CV$

is a fairly good approximation of the membrane ionic current. A similar calculation for repolarization gives a total membrane current of $2{\cdot}5 \times 10^{-7}$ A/cm², and a capacitative current of 120×10^{-7} A/cm². In this case, capacitative current is 50 times the membrane current.

The conclusion that I_m is negligible was verified by recording the time derivatives of action potentials elicited by threshold and maximal stimuli. With a maximal stimulus the latency of response was extremely short, and, since the conduction velocity was effectively infinite, I_m was close to zero. No difference in the derivatives during repolarization could be detected. Depolarizations could not be checked carefully because the artifacts were large, but there were no gross changes in slope. It is valid, therefore, to describe altered repolarization in unusual ionic media in terms of variations in membrane ionic current. Depolarization currents may be estimated in a similar manner, although less reliably.

Since the membrane ionic current can be estimated from $\dot{V}$, it will be useful to express this current in terms of its components, with the assumption that ionic current is carried only by Na^+, K^+, and Cl^- (Hodgkin & Huxley, 1952):

$$I_i = -C\dot{V} = g_{Na}(V-V_{Na}) + g_K(V-V_K) + g_{Cl}(V-V_{Cl}) \quad (3)$$

Since I_i and equilibrium potentials can be approximated, this equation serves to eliminate one of the unknown conductances. $\dot{V}$ also offers a convenient method of defining the phases of repolarization introduced by L. A. Woodbury, Hecht & Christopherson (1951). Thus, phase 1 corresponds to the region of the first maximum slope (not always present in frog ventricle); phase 2, to the succeeding minimum slope; and phase 3, to the final maximum slope.

RESULTS

A reduction in $[Na^+]_o$ has dramatic effects on the size and shape of the action potential (Fig. 1). Conduction block occurred when the substitution of choline for Na^+ had reduced $[Na^+]_o$ to 15% of normal or when the substitution of sucrose had reduced $[Na^+]_o$ to between 5 and 10% of normal. In both sucrose and choline solutions overshoot decreased as $[Na^+]_o$ declined, although the drop was somewhat less in the former. Reduction of $[Na^+]_o$ with choline substitution progressively decreased the duration of the action potential. Sodium concentrations in the 40–80% range, with sucrose substitution, shortened t_{AP} very little or occasionally elongated it; but in less than 50% $[Na^+]_o$, t_{AP} always shortened.

Depolarization

Effects of sodium

Membrane potential reversal. The mean overshoot of the *AP* recorded from 52 impalements was $20{\cdot}6 \pm 0{\cdot}9$ mV (S.E.). Ware, Bennett & McIntyre (1957) report an overshoot of 18·9 mV in frog ventricle. These two values are slightly higher than those for the same tissue reported elsewhere in the literature (13 mV, Woodbury *et al.* 1951; 14 mV, Trautwein & Zink, 1952; 16 mV, Kleinfield, Stein & Meyers, 1954). The variation of overshoot with $[Na^+]_o$ is shown in Fig. 2. Choline replaced Na^+ in equimolar quantities. From 120 to 40% $[Na^+]_o$ the changes in overshoot closely paralleled changes in V_{Na}. The lower line in Fig. 2 is drawn through the overshoot in 110 mM $[Na^+]_o$; the slope is 58 mV/decade. This line corresponds to a

$[Na^+]_i$ of 48 mM. Hajdu (1953) and Johnson (1957) reported $[Na^+]_i$ of 17·9 m-mole/l. fibre water and 14 m-mole/kg cells, respectively, in frog ventricle. These values correspond to a normal V_{Na} of 46 mV (upper line, Fig. 2) if the cells studied by Johnson are assumed to be 80 % water. The failure of the overshoot to reach V_{Na} probably means that the sum of the K^+ and Cl^- currents is appreciable at the peak of the action potential.

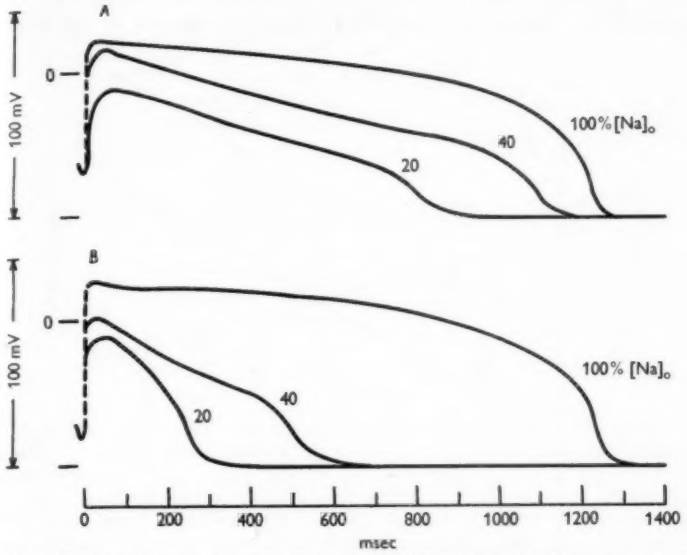

Fig. 1. Tracings of ventricular action potentials in 100, 40 and 20 % normal $[Na^+]_o$ with sucrose (*A*) and choline chloride (*B*) replacing NaCl. Temp. 15° C.

Evidence for appreciable Cl^- current is the finding that there was less decrease in overshoot with reduction of $[Na^+]_o$ when sucrose replaced NaCl (Fig. 1), than when choline replaced Na^+. With sucrose replacement the mean action potential amplitude in 88 mM-Na^+ was 9 mV higher than in 110 mM-Na^+ (overshoot was not measured in these experiments because there was drift in the recording system). Cl^- current will be considered further in the discussion.

The deviation of the overshoot toward zero potential when the $[Na^+]_o$ was less than 60 % (Fig. 2) might indicate a loss of intracellular Na^+. It can be seen from Fig. 2 that the overshoot when $[Na^+]_o$ was 30 % could have resulted from a loss of one third of the intracellular Na^+ during the 3 min perfusion period. This explanation is rendered less likely by the rapid time course of the change, the half-time being approximately 7 min. Hajdu (1953), who substituted maltose for NaCl, found a 30 min half-time

for Na^+ loss when $[Na^+]_o$ was 30 %. Alternatively, this effect is similar to, but not as drastic as, the lack of variation in overshoot observed by Coraboeuf & Otsuka (1956) when they altered the $[Na^+]$ in a solution bathing guinea-pig ventricle.

Depolarization rate. The maximum rate of depolarization in normal $[Na^+]_o$ averaged $30 \pm 1{\cdot}5$ V/sec (40 measurements). Ware *et al.* (1957) reported a mean of 33·9 V/sec in frog ventricle. The dependence of depolarization on $[Na^+]_o$ in a single impalement is shown in the insert in

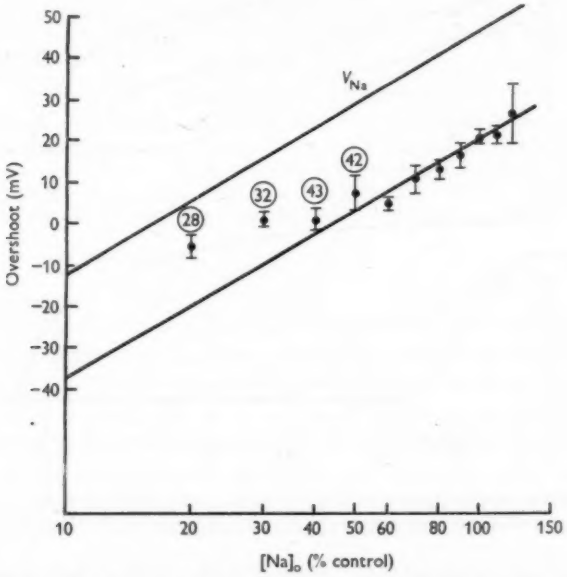

Fig. 2. Membrane potential overshoot as a function of log $[Na^+]_o$. Choline replacement of Na^+. Vertical bars are ± 2 S.E. Top line is V_{Na} calculated for $[Na^+]_i = 18$ mM. Bottom line is drawn through mean recorded overshoot in 110 mM-Na^+ with slope of 58 mV/decade and corresponds to $[Na^+]_i = 48$ mM. Numbers in circles are intracellular Na^+ concentrations necessary for Na^+ concentration potential to equal recorded overshoot. Stimulus rate 20/min, temp. 18° C.

Fig. 3. The maximum depolarization rate, although considerably lower than that in nerve or Purkinje tissue, was nevertheless markedly sensitive to $[Na^+]_o$. The depolarization rate varied enormously among fibres in a restricted area. The least-square line (Fig. 3) which passes through zero within one standard error indicates that Na^+ carries all the current necessary for depolarization. No consistent effects of $[Na^+]_o$ on resting potential were found.

Effects of potassium

Resting potential. The mean of 77 measurements of resting potential while the tissue was in normal Ringer's solution was $-79 \pm 1{\cdot}3$ mV. Ware *et al.* (1957) found a mean of $-84{\cdot}5$ mV. These values are considerably higher than those reported by others (-62 mV, Woodbury, Woodbury &

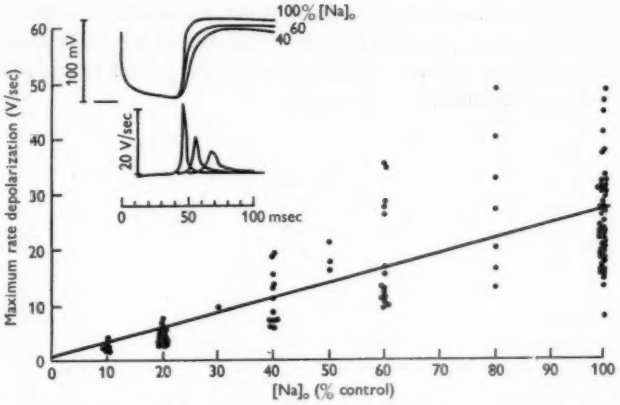

Fig. 3. Maximum rate of depolarization, measured electrically, as a function of $[Na^+]_o$. Line is a least-square fit of data: slope, $0{\cdot}257 \pm 0{\cdot}021$; intercept, $0{\cdot}7 \pm 1{\cdot}5$. Sucrose replaced NaCl.

Inset: depolarization in 100, 60, 40% normal $[Na^+]_o$ in a single impalement (upper tracings); electrical time derivative of depolarization (lower tracings); differentiating time constant = 50 μsec. Voltage and derivative calibrations shown on left, time base below. Upward deflexion preceding depolarization in upper tracings is stimulus artifact. Successive tracings are displaced slightly to the right for clarity.

Hecht, 1950; -65 mV, Woodbury *et al.* 1951; -64 mV, Trautwein & Zink, 1952; -58 mV, Kleinfield *et al.* 1954). According to Hajdu (1953) frog ventricular cells contain 89 m-mole K^+/l. cell water. If $[K^+]_o$ is assumed to be 2·5 mM, $V_K = -90$ mV. A discrepancy between V_r and V_K has also been observed in nerve and skeletal muscle (cf. Adrian, 1956). A possible explanation for this finding is that the process of active Na^+ extrusion in ventricle is similar to that of nerve, where an obligatory K^+ uptake accompanies active Na^+ extrusion (Hodgkin & Keynes, 1955).

The variation of V_r with $[K^+]_o$ is shown in Fig. 4 (solid dots). The line represents V_K if it is assumed that $[K^+]_i = 89$ mM. Except at high values for $[K^+]_o$ the curve is closely similar to that observed in other types of nerve and muscle fibre. The rate of change of V_r with log. $[K^+]_o$ was rather greater than 58 mV/decade at moderate concentrations and rather less at

high concentrations. Little significance can be attached to the latter observations, since few measurements in high $[K^+]_o$ were made. However, that the rate of change of V with $[K^+]_o$ was greater than 58 mV/decade over part or the range appears to be statistically significant. The cause of this effect is unknown.

Overshoot. Overshoot decreased as $[K^+]_o$ increased. Weidmann (1951) has shown that the overshoot in Purkinje tissue, as in nerve, depends on the initial V_r. The reduction of overshoot accompanying the decrease in V_r induced by a high $[K^+]_o$ found in this investigation was of a similar magnitude (Fig. 4, open circles). Altering both $[Na^+]_o$ and $[K^+]_o$ resulted in roughly additive changes in overshoot.

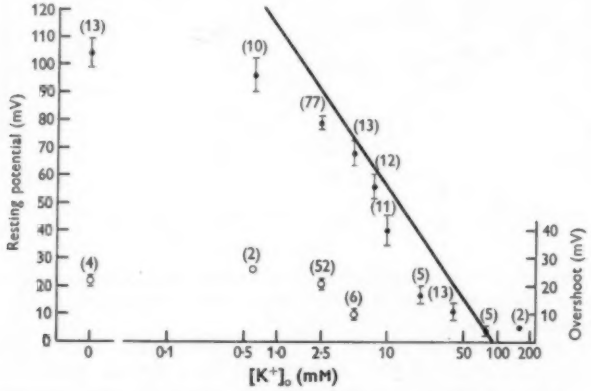

Fig. 4. Resting potential (●) as a function of $[K^+]_o$ on logarithmic scale; vertical bars are ±2 S.E. Line is K^+ concentration potential drawn through assumed $[K^+]_i$ of 89 mM with a slope of 58 mV/decade. Abscissa is broken to show resting potential in K^+-free perfusate. ○, action potential overshoot as a function of log $[K^+]_o$. Number of impalements shown in parentheses. Temp. 18° C.

Repolarization

Effects of sodium

Choline replacement. The duration of the action potential was dramatically shortened when $[Na^+]_o$ was reduced by replacing it with choline (Fig. 1*A*, Fig. 5, open circles). At 18° C the relationship between t_{AP} and $[Na^+]_o$ was linear between 110 and 20% normal $[Na^+]_o$. At 10° C the relationship was less linear, being more concave downward. Above 110% normal $[Na^+]_o$, t_{AP} was shortened. Other hypertonic solutions also shorten t_{AP}; e.g. in a perfusate made 25% hypertonic by addition of sucrose, t_{AP} decreased by 44% in 8 min.

Sucrose replacement. If sucrose was used to replace NaCl, t_{AP} varied

widely (Fig. 1*B*; Fig. 5, dots). On the average t_{AP} shortened very little until only 40 % normal $[Na^+]_o$ remained in the perfusate.

Probably at least two factors are involved in the differences between the effects of choline chloride and sucrose as substitutes for NaCl. (1) The choline may well have had an acetylcholine-like effect of increasing K^+ efflux. Thus, Hoffman & Suckling (1953) showed that acetylcholine dramatically decreases the duration of the action potential in mammalian

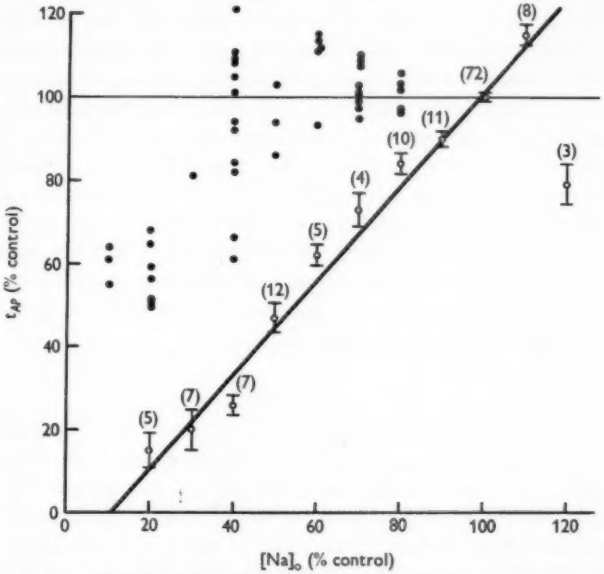

Fig. 5. Action potential duration (t_{AP}) as a function of $[Na^+]_o$ with sucrose (●) and choline chloride (○) replacing NaCl. Each point represents one action potential in sucrose solution. Line is least-square fit of data in choline solutions, excluding values for 120 % normal $[Na^+]_o$. Stimulus rate 20/min, temp. 15° C.

auricular fibres. Trautwein *et al.* (1956) have published evidence that acetylcholine increases the conductance of frog auricular fibres and Harris & Hutter (1956) have shown that both influx and efflux of ^{42}K are increased in frog sinus venosus by acetylcholine. Hajdu (1953) states that choline chloride exerts an acetylcholine-like effect on the staircase phenomenon by increasing the net loss of K^+, and that this effect is abolished by atropine. (2) The fact that t_{AP} was often prolonged when sucrose replaced a portion of the NaCl in the bathing solution suggests that Cl^- normally carries an appreciable current in normal repolarization. Thus, when both $[Cl^-]_o$ and $[Na^+]_o$ were reduced, t_{AP} was greater because the overshoot was not

greatly affected and because a longer time is needed if K^+ current alone recharges the membrane. The duration of the action potential changed in the same manner with sucrose substitution as with choline substitution if the ventricle was first perfused with a choline solution. This result indicates an irreversible acetylcholine-like effect.

Either choline or sucrose substitution caused a marked reduction in the plateau voltage. The effect was expected on the supposition that influx of Na^+ is the cause of depolarization and the maintenance of the plateau. The exaggeration of the slope of the first phase was also expected, since the peak of the action potential is nearer V_{Na} and a decrease in inward Na^+ current will have less effect on V at this time than later when some repolarization has occurred. However, the reason for the development of a first phase inflexion in low $[Na^+]$ is not evident (see Discussion). It is also of interest to compare with the effects of progressively reduced $[Na^+]_o$ the effects of progressively increased hyperpolarizing currents, applied for the duration of the action potential (Cranefield & Hoffman, 1958), and those of vagal stimulation (Hoffman & Suckling, 1953; Woodbury & Brady, 1956). In all three cases the first phase becomes more and more prominent and the second phase less and less prominent until all three phases finally fuse into an uninflected repolarization.

Effects of potassium

The shortening of t_{AP} with increasing $[K^+]_o$ (Brady & Woodbury, 1957; Weidmann, 1956*b*) is unexpected from the point of view that repolarization is accomplished by a net outward current of K^+ driven by $(V-V_K)$. Although a change of $[K^+]_o$ has little effect on the currents of phases 1 and 2, such a change probably does alter membrane conductance to K^+ or Cl^- during phase 3, because the maximum rate of repolarization is roughly proportional to $[K^+]_o$ (Brady & Woodbury, 1957).

Effects of frequency of excitation on repolarization

Several investigators have found a monotonic relationship between t_{AP} and stimulus interval (Siebens, Hoffman, Gilbert & Suckling, 1951; Woodbury *et al.* 1951; Trautwein & Dudel, 1954). This effect has been confirmed and extended in these experiments. The duration of the action potential reached a plateau for t_s greater than 3 sec at 18° C. For a t_s less than 0·5 sec the *AP* markedly undershot and became erratic in amplitude. It is difficult to excite the ventricles at rates higher than 80–90/min unless the stimulus rate is increased very gradually; i.e. without interruption of the stimulus train. If the ventricle fails to respond to even one stimulus, excitation occurs at a submultiple of the applied stimulus frequency. In reduced $[Na^+]_o$ the shortening of t_{AP} was even more pronounced as the

stimulus rate was increased. In 50% normal $[Na^+]_o$, excitation rates up to 300/min were readily attained.

Superimposability of action potentials

Figure 6 illustrates the large degree to which the later phases of repolarization are superimposable when excitation rate is changed. The tracings were adjusted for best superimposability mainly by a simple shift in time. In some instances, particularly at 50% $[Na^+]_o$, adjustments in

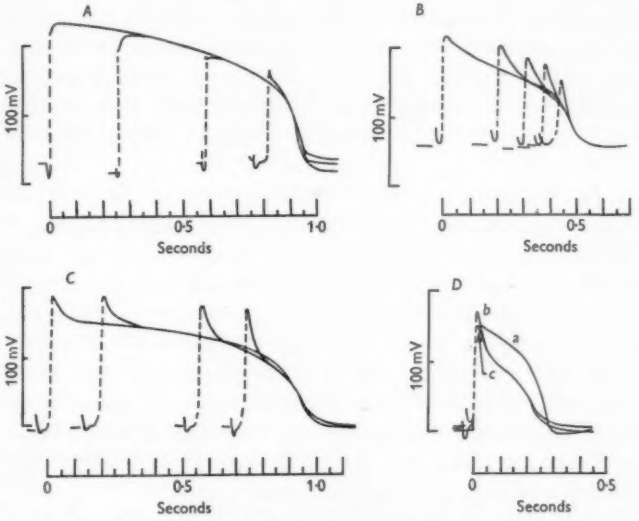

Fig. 6. Tracings of action potentials adjusted for best fit of repolarization phases. Zero base lines of tracings do not necessarily correspond. *A*, tracings in normal Ringer's solution, stimulus rates 25, 50, 120, 130/min respectively, left to right. *B*, tracings in 50% normal $[Na^+]_o$ at stimulus rates 25, 55, 90, 125, 230/min, respectively. *C*, tracings in 50% normal $[Na^+]_o$, K^+-free at stimulus rates 20, 50, 85, 120/min. *D*, superimposed tracings: *a*, normal Ringer's solution, 128/min; *b*, 50% $[Na]_o$, 0% $[K^+]_o$, 122/min; *c*, 50% $[Na]_o$, 27/min; choline replacement of Na^+ in all cases.

the vertical position of a few millivolts were also required. In normal Ringer's solution the repolarization phase of the shorter action potentials appeared to start from a lower reversal potential, but traversed a final time course nearly identical with that of longer action potentials. At relatively high rates V_r decreased, possibly because of K^+ accumulation outside the membrane during activity. The reduction in V_r would reduce the overshoot, which in turn would decrease t_{AP}. This possibility is also suggested by Carmeliet & Lacquet's (1958) finding that the variation of t_{AP} with rate

of excitation shows the same exponential relation as the variation of t_{AP} with $[K^+]_o$. On the other hand, since the maximum rate of depolarization depends on $[K^+]_o$, at least in frog ventricle (Brady & Woodbury, 1957), shortening of t_{AP} due to K^+ accumulation is not superimposable (see Discussion).

As Fig. 6 clearly demonstrates, superimposability holds only when t_{AP} is changed by altering t_s. Changes in t_{AP} produced by varying $[Na^+]_o$ or $[K^+]_o$ also change the shape of the *AP*. The lower right-hand tracings in Fig. 6 show the form of repolarization when action potentials of similar durations were produced by different means. It is of interest that *b* and *c* have almost identical contours with more than a fourfold difference in excitation rate. The sizable fall in plateau voltages in *b* and *c* (lower right) suggests the presence of a slowly decreasing inward Na^+ current in early repolarization, since these potentials were recorded at a lower $[Na^+]_o$ than that influencing *a*.

DISCUSSION

The process of depolarization is probably the same in the heart as it is in the squid axon. The main problem in heart muscle, therefore, is to describe the process of repolarization. A hypothesis is developed here to explain qualitatively most of the observations on the repolarization of cardiac action potential with only minor modifications of the Hodgkin-Huxley theory. This hypothesis is quite speculative: the facts are in accord with it but they do not serve to eliminate a number of alternatives. Weidmann's (1951) early speculations were similar but less detailed. More recently, Coraboeuf, Zacouto, Gargouil & Laplaud (1958), and Shanes (1958) have speculated along much the same lines.

Depolarization

There is considerable evidence that Na^+ is the depolarizing agent in frog ventricle. (1) Excitability is abolished in Na^+-free solutions. (2) The rate of depolarization and overshoot depend on $[Na^+]_o$. Although the maximum rate of depolarization varied greatly from fibre to fibre, the reduction of $\dot{V}_d$ in low $[Na^+]_o$ is convincing in a single impalement. No additional ions are needed to carry the required depolarizing current, since the curve relating $\dot{V}_d$ to $[Na^+]_o$ (Fig. 3) passes through the origin. If all the depolarizing current is carried by Na^+, peak g_{Na} can be estimated by setting $g_{Cl} = g_K = 0$ in equation (3); $g_{Na} = -C\dot{V}_d/(V-V_{Na})$. Assuming a high value for C (30 μF/cm^2), $\dot{V}_d = 30$ V/sec; $V = -40$ mV and $V_{Na} = 50$ mV; then $g_{Na} = 10$ mmho/cm^2. Hodgkin & Huxley (1952) found a maximum g_{Na} in squid axon of about 25 mmho/cm^2; even higher values have been reported by Cole (1958). Thus, no unreasonable magnitude of g_{Na} is required in the heart.

The failure of the overshoot to reach V_{Na} is most readily explained by assuming that a considerable inactivation of g_{Na} has occurred at the peak of the action potential; i.e. $g_K + g_{Cl}$ is appreciable at this time. Equation (3) can also be used to estimate $g_{Na}/(g_K + g_{Cl})$ at the peak of the action potential where $\dot{V} = 0$ and $I_i = 0$. If it is assumed that $V_K = V_{Cl} = V_r$ and that $V_r = -80$ mV, $V_a = 20$ mV and $V_{Na} = 46$ mV, then $g_{Na}/(g_K + g_{Cl}) = 4$. The same ratio in squid giant axon is about 6 (Hodgkin & Huxley, 1952). In squid axon the ratio is as low as this because g_K has risen appreciably by the peak of the action potential, although g_{Na} is still near its peak value. Since there are indications that g_K does not increase and may in fact decrease in heart tissue following depolarization (Weidmann, 1951), the low $g_{Na}:(g_K + g_{Cl})$ ratio of the ventricle at the peak of the action potential can be attributed to inactivation of g_{Na} rather than to an increase in g_K (Johnson, Robertson & Tille, 1958).

As mentioned previously, Coraboeuf & Otsuka (1956) found that a reduction of $[Na^+]_o$ well below 25% of normal had little effect on the overshoot of the action potential of guinea-pig ventricle. However, excitability was lost in 5% solutions in about 20 min. Délèze (1959) confirmed these observations on the overshoot, but also found that $\dot{V}_d$ fell to one third of normal in 25% $[Na^+]_o$. This observation is strong evidence that Na^+ is the depolarizing agent. The lack of effect on overshoot is puzzling, but similar to the effects of a $[Na^+]_o$ of 50% or less on frog ventricle (Fig. 3). Possible explanations are: (1) the rate of rapid inactivation of g_{Na} is decreased in low $[Na^+]_o$ solutions, and (2) the degree or rate of fall of g_K following depolarization is affected by $[Na^+]_o$.

Repolarization

A number of facts hint at the basic mechanism of repolarization. (1) Action potentials of different durations are approximately superimposable if shifted in time. (2) The duration of the action potential and the voltage of phase 2 depend directly on the excitation interval and the $[Na^+]_o$. (3) In an experimental approximation of total slope conductance (G) during repolarization in Purkinje tissue, Weidmann (1951) found that G falls to about one third of its diastolic value at the end of the second phase and returns to normal with rapid repolarization. The results of Coraboeuf *et al.* (1958) on guinea-pig ventricle were similar except that G was greater throughout the plateau than at rest.

The first two points indicate the dependence of changes in chord conductance on time and voltage, whereas the third drastically limits the characteristics of the chord conductance changes which may be involved. The superimposability of the trailing edges of action potentials indicates that the chord conductances are voltage-dependent in the superimposable

range. On the other hand, the slow fall of the potential during the plateau and the variation in plateau length with stimulus interval suggest that the conductance changes responsible for the maintained depolarization are time-dependent. These inferred conductance changes are a qualitative description of the behaviour of g_{Na} in squid axon during activity (Hodgkin & Huxley, 1952), although the time scale for them is much slower.

The following postulates concerning altered conductance during activity, and the manner in which they explain many of the observed phenomena, will aid discussion of the similarities between action potentials in cardiac muscle and in squid giant axon. (1) Following depolarization, g_{Na} follows a double exponential decay—a large rapid fall (order of milliseconds) succeeding the high g_{Na} of depolarization and a small, slow decline (order of seconds) responsible for the plateau phase. (2) Following repolarization, available g_{Na} is reconstituted over about the same time course the large, rapidly decaying part recovering quickly and the small, slowly decaying part recovering slowly. (3) Chloride conductance is constant throughout activity. (4) Sudden depolarization causes a reduction in g_K over a rather long time; i.e. the cardiac membrane exhibits anomalous rectification similar to that of skeletal muscle (Hodgkin & Horowicz, 1959). The most direct evidence for a fall in the g_K in heart is the absence of an increase in K^+ efflux when the excitation rate is increased (Brady, unpublished). If this observation can be taken at its face value, I_K must decrease to nearly zero during the plateau to compensate for the K^+ efflux required to repolarize the membrane. Another reason for supposing that g_K decreases during activity is that Na^+–K^+ exchange is reduced, so that the load on the Na^+–K^+ pump is reduced. However, a fall in g_K is not an essential element of the hypothesis.

Hypothetical events of repolarization

Following a threshold depolarization, g_{Na} and V increase regeneratively. Na^+ conductance reaches its peak value at or shortly after the inflexion point of the rising phase and has fallen to the comparatively low value of four times $(g_K + g_{Cl})$ at the peak of the action potential. The rapid phase of inactivation is probably near completion at this time. During the plateau, inward Na^+ current must very nearly equal the outward K^+ and Cl^- currents, since $\dot{V}$ is nearly zero. The voltage falls slowly because g_{Na} is slowly inactivating, with a consequent decrease in inward Na^+ current. Rapid repolarization begins when V reaches a range in which some conductance varies rapidly with V. Since g_K is presumed to have decreased with depolarization, it probably is rapidly increasing at this time; thus the net outward current and the rate of repolarization increase. Since there is

some residuum of slowly inactivating g_{Na}, it must also be supposed that g_{Na} is turned off by repolarization.

The absolute refractory period ends at about the third phase inflexion point. Inactivation of g_{Na} must be maximum at this time. As repolarization proceeds the rapid part is quickly reactivated, for an action potential evoked when V is about 10 mV above the resting value is near its full height. However, the slowly reactivating part is still small, for an action potential elicited at this time has a full height but a short duration. Thus, stimuli following repolarization will produce successively longer action potentials as the slow fraction recovers. Thus, if slow reactivation is exponential, the t_{AP}–t_s curve should also be exponential. Carmeliet & Lacquet (1958) give time constants of 1·0–1·5 sec for the t_{AP}–t_s curve in frog.

Membrane currents and conductances

Slope conductance. The limitations imposed by Weidmann's results can be inferred from the relationship between chord conductance as defined by equation (3) and slope conductance as estimated with applied current pulses. Total slope conductance, G, is defined as the rate of change of ionic current with respect to V; i.e. $G = \partial I_i/\partial V$ at $I_i = 0$. Differentiation of equation (3) gives:

$$G = g_{Na} + \frac{\partial g_{Na}}{\partial V}(V - V_{Na}) + g_K + \frac{\partial g_K}{\partial V}(V - V_K) + g_{Cl}. \tag{4}$$

Weidmann estimated G by measuring the change in membrane voltage (ΔV) produced by an applied current (I_o). The quantity $I_o/\Delta V$, after correction for cable properties, is an approximation of $\partial I_m/\partial V$. Since I_m is the total membrane current, the relationship between $\partial I_m/\partial V$ and G can be obtained by differentiating equation (1) with respect to V. Thus

$$\frac{\partial I_m}{\partial V} = \frac{C\partial \dot{V}}{\partial V} + G \quad \text{or} \quad G = \frac{\partial I_m}{\partial V} - C.\frac{\partial \dot{V}}{\partial V}. \tag{5}$$

That is, to learn the value of G, the portion of the applied current flowing through the membrane capacitor must be subtracted from $\partial I_m/\partial V$. The results of Cranefield & Hoffman (1958), and Tanaka (1959), indicate that the $C\partial\dot{V}/\partial V$ term is no more than about 20 % of the total value of $\partial I_m/\partial V$ during repolarization in cat ventricle and frog atrium. The $\partial\dot{V}/\partial V$ term will henceforth be neglected.

Since by postulate g_{Na} increases with V, $\partial g_{Na}/\partial V$ is a positive quantity; therefore, $(V - V_{Na})(\partial g_{Na}/\partial V)$ is always negative. Thus, slope Na^+ conductance is always less than or equal to chord conductance, since $G_{Na} = g_{Na} + (V - V_{Na})(\partial g_{Na}/\partial V)$. During rapid depolarization when $\partial g_{Na}/\partial V$ and $(V - V_{Na})$ are large, it is quite possible that G_{Na} becomes negative. Even if g_K

remained constant at its resting level during activity, G could still follow a time course similar to that found by Weidmann, despite the fact that total chord conductance would be greater than normal at all times. Similarly, if depolarization decreases g_K, G_K is less than g_K. Further, if g_K is increasing while g_{Na} is decreasing, and if g_{Cl} is not too large, total G might become negative.

Chloride current. If Cl^- is not actively transported and the membrane is readily permeable to Cl^-, V_{Cl} should be close to the average voltage ($\bar{V}$) in a steadily beating heart, for $t_s = 3$ sec $\bar{V}$ approximates to -50 mV. A V_{Cl} of -50 mV should speed early and retard late depolarization and repolarization. The smaller reduction in V_a in low-NaCl solutions relative to low-Na^+ solutions (Fig. 1) is consistent with this view for depolarization. The longer t_{AP} in sucrose substitutions could result from a relatively higher overshoot and a higher plateau voltage, since Cl^- current is not available to help repolarization. Recent experiments with Cl^- substitution indicate that g_{Cl} in heart tissue is not as large as in skeletal muscle, but is of the order of 0·2 g_r. Hutter & Noble (1959; O. F. Hutter, personal communication) estimate g_{Cl} as 0·23 g_r in mammalian Purkinje fibres.

Sodium and potassium currents. Hodgkin & Horowicz (1959) point out that the behaviour of the skeletal muscle fibre membrane is probably diphasic in time, i.e. the falling phase of the action potential is brought about by a delayed increase in g_K, whereas if the depolarization is maintained, g_K eventually decreases below the resting value. It is tempting to suppose that the fall in g_K following an abrupt depolarization is comparatively slow in cardiac tissue. Such a supposition could explain the double peaks of the action potential in the cat ventricle (Cranefield & Hoffman, 1958) and possibly the appearance or exaggeration of the first phase in the action potential of a frog ventricle when $[Na^+]_o$ is low (Figs. 1, 6). The first maximum in the cat ventricular action potential is brought about by rapid g_{Na} inactivation. As g_{Na} falls, I_K exceeds I_{Na} and $\dot{V}$ becomes negative. However, g_K is also falling rather slowly and, at the completion of the rapid inactivation of g_{Na}, I_K falls below I_{Na} momentarily, making $\dot{V}$ positive.

The effects of a low $[Na^+]_o$ on the action potential are roughly what would be expected from a decrease in inward current. However, the occurrence or accentuation of a first phase when $[Na^+]_o$ is subnormal is difficult to explain. In fact, the development of an inflexion point in the plateau phase where the slope is minimum seems to require the postulation of a process in addition to slow inactivation. Reduction in V_{Na} alone should steepen the slope at all times in proportion to the change in $(V-V_{Na})$ between the two solutions. The widely disparate time courses of the first phase when NaCl is replaced by choline chloride and by sucrose (Fig. 1),

and the fact that the point of minimum slope occurs at about the same voltage in the two cases, suggest that a voltage-dependent rather than a time-dependent process is involved. On the other hand, if sucrose replacement markedly reduces total membrane slope conductance, the longer time course could be explained as well by a time-dependent phenomenon such as the delayed fall in g_K following depolarization that was mentioned above. A voltage-dependent phenomenon that might explain the occurrence of a first phase is a dip in the I_K–V curve at the voltage of the inflexion point of the second phase. Such a dip might be obscured by the high I_{Na} and I_{Cl} in normal $[Na^+]_o$ and $[Cl^-]_o$. However, the dip might noticeably affect V when I_{Na} is reduced by sucrose replacement of NaCl or if I_K is increased by choline replacement of Na^+. Freygang & Adrian (1960) found that the I_K–V curve for frog muscle fibres soaked in a 100 mM-K choline sulphate solution had a dip at V–V_K = 60 mV. They also found that the P_K, (V–V_K) curves are about the same shape regardless of $[K^+]_o$. Thus the dip in the I_K–V curve may well occur at a normal value for $[K^+]_o$. A dip at V–V_K = 60 is in approximately the right place.

Superimposability. As mentioned above, superimposability suggests that conductance changes are voltage-dependent and that the cause of the shortening of t_{AP} with t_s is a reduction in the amount of slow g_{Na} available; i.e. the decreased diastolic interval limits the reconstitution of g_{Na}. This quantity determines the height of the plateau and thus the duration. That t_{AP} depends on plateau rather than on overshoot can be seen from the two left-hand action potentials in Fig. 6, lower left. Although they have the same peak height, their durations differ by 200 msec. Thus the duration of repolarization is accurately determined by the membrane voltage present 100–200 msec after the upstroke.

The phenomenon of superimposability does not severely limit the mechanisms underlying repolarization, as has been demonstrated in attempts to predict the cardiac action potential from mathematical formulations of the postulated conductance changes. Several formulations quite successfully predict the time course of the cardiac action potential, the change in t_{AP} with stimulus interval and the superimposability of these action potentials. An example is shown in Fig. 7*B*. The superimposed action potentials of different lengths represent solutions of the differential equation (3) for various initial values of the slow component of g_{Na}. Comparison of Fig. 7*B* with Fig. 6, upper left, shows a remarkable similarity between the two families of curves. However, this particular formulation must be rejected because slope conductance assumes large negative values during the third phase.

Careful examination of the results obtained by Weidmann (1951);

Cranefield & Hoffman (1958) and Tanaka (1959) leads to the conclusion that slope conductance is positive throughout repolarization. Consideration of the definition of slope conductance (equation (4) and previous line) shows that if G is positive the system is stable; i.e. $\dot{V}$ approaches zero at that voltage where the $I_i (= -C\dot{V})$, V curve passes through zero. If G is negative, the voltage where $I_i = 0$ is an unstable equilibrium point. A small displacement, say in the form of an applied current, will cause a regenerative increase in $\dot{V}$, i.e. threshold behaviour. A small current pulse applied to a membrane will produce a voltage change with an uninflected time course if G is positive. Upon cessation of the current the voltage will return to near its original path. On the other hand, if G is negative an applied pulse will produce an inflected voltage change and radically alter the time course of the subsequent potential change. Experimentally, a depolarizing current, even a small one, changes the sign of $\dot{V}$. This shows that during repolarization V stays near its stable equilibrium voltage, which is slowly decreasing. This behaviour is different from that of the squid giant axon, where the slowly increasing g_K following the upstroke abolishes the unstable equilibrium (threshold) point and the stable point near V_{Na}, and repolarization proceeds rapidly from near V_{Na} along a positive slope I_i–V curve toward the sole remaining, nearly stationary stable point near V_K. This is not to say that a sufficiently strong repolarizing current will not restore the cardiac cell membrane to the resting state. Weidmann (1951) and Cranefield & Hoffman (1958) have produced early repolarization by applied currents, but only after bringing the membrane potential to a value near the resting level for a considerable period. In this case the transition or threshold voltage is outside the range of those passed through in normal repolarization.

Mathematical formulation. Although several mathematical descriptions of the ionic currents quite accurately predict the shape of the action potential, none have given a positive slope conductance throughout repolarization. The most successful model developed was derived by supposing that fast repolarization is largely due to K^+ and that g is less than or equal to g_r. This assumption requires that the resting membrane time constant (τ) be only about 5 msec. With assumed values for V_K and $g_{Na,r}$ and measured values for $\dot{V}$, I_K was calculated from $I_K = -C\dot{V} - g_{Na,r}(V - V_{Na})$. For simplicity of calculation, g_{Cl} was taken as zero. Values of $g_K = I_K/(V-84)$ are well described by the equation

$$g_K = \left(0{\cdot}2 + \frac{0{\cdot}78}{1+\exp{(V+63)/6}}\right) g_r$$

over the voltage range -84 to -54 mV. At larger voltages the calculated I_K diverges from the estimated value. The excess is attributed to an increasing I_{Na}. I_{Na} values at each voltage were corrected for inactivation by multiplying by $\exp(t/1000)$, where t is the time after depolarization at which the action potential passed through that voltage and 1000 msec is the assumed time constant of inactivation. The corrected I_{Na} was divided by $V-40$ to obtain g_{Na}; g_{Na} is moderately well described by $g_{Na} = (0{\cdot}70 \exp V/27 + 0{\cdot}033)\, g_r$. Since this slowly inactivating component of g_{Na} has a slow voltage-dependence, a separate highly voltage-dependent term for the rapidly inactivating (5 msec time constant) component was

added to g_{Na}. These expressions for g_{Na} and g_K can be substituted into equation (3) to obtain a differential equation which describes V as a function of time:

$$-\tau\dot{V} = \left(\frac{2\exp(-t/5)}{1+\exp\{(-V-50)/6\}} + \frac{0{\cdot}7\exp(V/27)}{\exp(t/1000)} + 0{\cdot}033\right)(V-40) + \left(\frac{0{\cdot}78}{1+\exp\{(V+63)/6\}} + 0{\cdot}2\right)(V+84). \quad (6)$$

Approximate solutions of this equation have been obtained with an IBM 650 digital computer. Figure 7*A* is a plot of V and G as a function of time with $V = -50$ at $t = 0$. Zero time is the time at which both slow and fast inactivation begin. A real action potential,

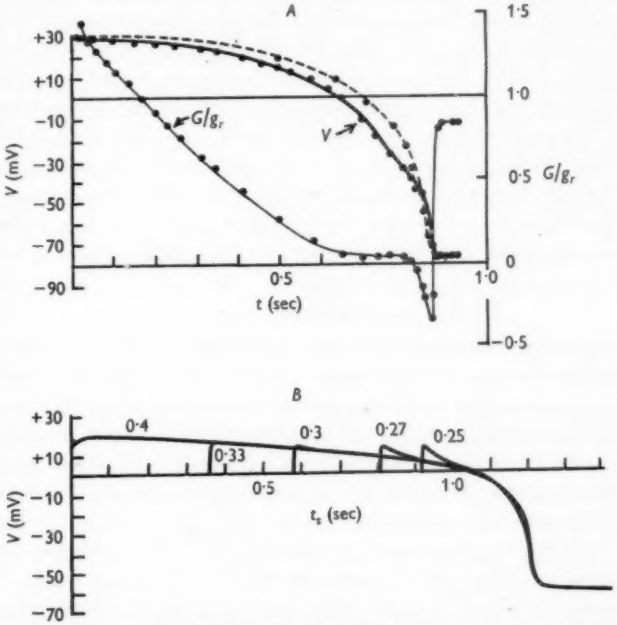

Fig. 7. *A*. Action potential (heavy line) and total slope conductance (light line) computed by integration of equation (7). Broken line, action potential of frog ventricle scaled to same size and duration. *B*. Superimposed calculated action potentials for indicated values of g_{Na}/g_r, showing simulated changes in t_{AP} with t_s. The action potentials are the solutions of a somewhat different formulation of equation (7).

scaled to the same amplitude and duration, is shown for comparison. The computed and real action potentials are quite similar in shape and duration. The low voltage of the third phase inflexion and its rather steep slope (1 V/sec), which are typical of frog ventricle, are accurately predicted. The calculated potential is not sufficiently rounded in the region of maximum curvature before the third phase inflexion. It seems probable that adjustment of the constants or a better fit of the I_{Na}–V curve would rectify this deficiency.

The major defect is that G becomes negative during the third phase, although its values are appropriate at other times. It seems unlikely that minor modifications could prevent

this difficulty, since G_K must be negative in this region in order to fit the experimental data. Negative G_K reflects a dip in the I_K–V curve of the type postulated above to account for the occurrence of a first phase in low $[Na^+]_o$. However, the equation for I_K has a dip rather closer to V_K than was found by Freygang & Adrian (personal communication). Thus, the requirement of positive G throughout repolarization severely limits the types of current-voltage curves which can be used. In fact, the restrictions are so great that it seems quite possible that there is at least a small negative G late in repolarization in heart muscle. Such a phenomenon could easily be missed experimentally. The equation for I_K can be modified slightly to account for the lack of increase in K efflux during activity (Brady, unpublished). If the constant part of g_K ($0{\cdot}2\ g_r$) is attributed to Cl^-, predicted I_K is near zero at plateau voltages and an appreciable K^+ efflux would occur only during depolarization and repolarization. A rough calculation shows that such a complete turn off of g_K is necessary to account for Brady's results, on the assumption that K^+ influx is not altered by activity.

Weidmann (1951), Shanes (1958) and Coraboeuf *et al.* (1958) have proposed explanations of the cardiac action potential generally similar to the hypothesis presented here. Weidmann and Shanes have noted the possibility of 'incomplete inactivation' as an explanation for the plateau, and Shanes suggested that g_K decreases with increasing depolarization. Shanes's explanation of the t_{AP} versus t_s curve is not clear to us, but seems to be based on changes in g_K during the previous diastolic period.

Concluding remarks

These interpretations are largely speculative. The deductions and inferences with respect to mechanisms are necessarily indirect, and voltage clamp type or similar experiments are necessary to test critically the proposed mechanisms. As far as possible the postulated mechanisms of heart activity have paralleled the known mechanisms of the squid giant axon. Fortunately the available evidence supports the required modifications. There is no direct evidence to support the postulate that there are fast and slow g_{Na} inactivation in heart, but this postulate so simply explains the most puzzling aspects of the cardiac action potential as to make it a useful concept. Cole (1958) has reported that hyperpolarization produces a small degree of activation of g_{Na} lasting many minutes in the squid giant axon. Together with an ancillary assumption about g_K—that it decreases during activity—the slow component of g_{Na} qualitatively explains: (1) the plateau and prolonged duration of the action potential, (2) the superimposability of action potentials whose durations are altered by stimulus rate, (3) the dependence of the duration of the action potential on time between successive stimuli and (4) the effects of a low $[Na^+]_o$ on repolarization.

Some doubts that the Na^+–K^+ theory of Hodgkin and Huxley is applicable to cardiac tissue have been expressed by Tasaki & Hagiwara (1957). They base their conclusions on the similarity between the action potential of the tetraethyl ammonium (TEA)-treated squid giant axon

and the cardiac action potential. The analogy is probably not valid, since the threshold for repolarization of an axon treated with TEA is above the resting level. Repolarization is thus brought about by a transition through a region of negative slope conductance. Additionally, the hypothesis developed here *does* explain qualitatively the major properties of the cardiac action potential within the framework of the Hodgkin–Huxley Na^+–K^+ theory.

SUMMARY

1. In frog ventricle the overshoot of the membrane potential and the maximum rate of depolarization of the action potential depend directly on the external concentration of Na^+.

2. The behaviour of the membrane resting potential is similar to that of nerve and skeletal muscle with respect to alterations in the external concentration of K^+. Overshoot diminishes when external K^+ concentration is supranormal about as expected from voltage-dependent inactivation of Na^+ membrane chord conductance.

3. The duration of the action potential is very dependent on the external concentration of Na^+ when either sucrose or choline is substituted for Na^+, but is most strongly so when choline is present.

4. The later phases of repolarization are superimposable when duration is altered by changing the stimulus rate, suggesting that voltage-dependent conductance changes are responsible for fast repolarization.

5. The principal differences between the known properties of the squid giant axon and the properties of the heart postulated here are: (*a*) the inactivation and activation processes in heart have slow (order of seconds) as well as fast (order of milliseconds) components and (*b*) depolarization decreases rather than increases K^+ membrane chord conductance.

6. The significance of slope conductance measurements is discussed. The effects of external currents on repolarization indicate that this process is brought about by a slowly decreasing stable equilibrium voltage. This behaviour is markedly different from that in the squid giant axon.

7. It is concluded that the Hodgkin–Huxley theory, suitably modified, accounts for the major properties of the cardiac action potential.

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BODY SIZE, BODY TEMPERATURE AND AGE IN RELATION TO THE METABOLIC RATE OF THE PIG IN THE FIRST FIVE WEEKS AFTER BIRTH

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In the period following the birth of the young pig there are in the normal animal at least three apparent factors which could influence the metabolic rate. First, the pig grows rapidly, and heat production would be expected to increase with increasing size of the animal. Secondly, the deep body temperature would be expected to influence metabolic processes, either directly or reflexly; related to this, the new-born pig's metabolic rate rises during the first two days after birth, and there is an associated rise in rectal temperature (Mount, 1959). Thirdly, the increasing age of the pig could be indicative of physiological development which would influence the metabolic rate; in this connexion Gelineo (1959) has reviewed the development of homoeothermy in mammals.

The question which arises, then, is how the growing pig's oxygen consumption rate is related to each factor independently of the other two. The present investigation is an attempt to answer this question. The method has been to apply multiple regression analysis to oxygen consumption rates of pigs during the first five weeks after birth in relation to body weight, rectal temperature and age. Some of these results have been the subject of a preliminary communication (Mount & Rowell, 1960).

METHODS

The experiment involved 98 Large White pigs of both sexes. The farrowing house in which they were born was fitted with heated creeps and the ambient air temperature varied between 7 and 15° C. Observations were made at two temperatures, 4° and 30° C, so that relations could be studied at a high and a low mean metabolic rate. The investigation was carried out in three parts, differing essentially only in the ambient temperature at which the observations were taken and in the age of the pigs. The means and ranges of age and body weight for the three parts are included in Table 1; the three parts will be referred to as 30° C first week, 4° C first week and 30° C 1–5 weeks.

Oxygen consumption, as dry gas at s.t.p., was used as the measure of metabolic rate and its rate was determined for each pig singly in a closed-circuit apparatus. Measurements were taken as soon as temperature equilibrium was reached in the metabolic chamber, which was usually within 1 hr of the introduction of the animal. The pigs received no previous treatment and were unrestrained in the chamber; the period of measurement was at least half an hour.

Rectal temperature and body weight were measured immediately before and after the determination of oxygen consumption rate and the averages of the two figures were used in the analysis of the results. A description of the methods for measuring oxygen consumption and rectal temperature has been given elsewhere (Mount, 1959).

Biometrical methods

The relation between oxygen consumption rate and body weight, rectal temperature and age was examined by means of multiple regression techniques (Fisher, 1958). The advantage of this procedure over the regression methods in which each variable is studied separately is that it is possible to study the regression of oxygen consumption rate on body weight, for example, independently of rectal temperature and age; that is, the regression as though all pigs had the same rectal temperature and were the same age. The regression coefficients so obtained are called partial regression coefficients. The method allows an estimate to be made of the amount of variation in oxygen consumption rate between pigs which can be accounted for by variations in body weight, rectal temperature and age. Much of the variation which is not so accounted for (the 'residual variance') is probably due to real differences between

TABLE 1. Means and ranges of oxygen consumption rate, body weight, rectal temperature and age for pigs in the first 5 weeks after birth

Ambient temperature and age	No. of pigs	Oxygen consumption rate (ml./min)	Body weight (kg)	Rectal temperature (°C)	Age
30° C first week	61	30·2 (15·7–55·2)	1·69 (0·95–3·37)	39·1 (37·8–39·8)	2·79 days (5 hr–6·25 days)
4° C first week	18	58·3 (32·5–93·8)	1·58 (1·07–2·72)	38·4 (36·5–39·4)	3·32 days (1 hr–6·83 days)
30° C 1–5 weeks	19	54·4 (29·9–70·5)	4·79 (2·88–7·60)	39·5 (38·6–40·2)	21·32 days (10–37 days)

pigs rather than errors of measurement. Thus, for example, different pigs of the same weight, rectal temperature and age may not have the same surface area or the same thermal conductance from body core to surface and so may not have the same rate of heat loss.

In all the analyses to be described linear relations were assumed. The validity of this assumption was tested in two ways. First, the partial regression coefficients for the younger pigs and the older pigs were compared. These coefficients would be about the same size if and only if the relations were linear. The results given in the next section suggest that log. (oxygen consumption rate) is approximately linearly related to log. (body weight) and rectal temperature, but that the relation with age is curvilinear. The second method of testing linearity was to compare the residual variances when different transformations were applied to the variables; the transformation resulting in a straight line which fits the results most closely will result in the smallest residual variance about the line (after adjusting the variances to the same units).

Six analyses were compared, the different functions of the dependent and independent variables being given in Table 2, along with the residual standard deviation for each analysis. These residual standard deviations measure the variation in oxygen consumption rate between pigs of the same weight and age and with the same rectal temperature; they are remarkably similar and suggest that there is little to choose between the analyses on purely statistical grounds for pigs up to 5 weeks old. The indices for body weight which were used in analyses 2 and 3 are based on the assumption that the animal's surface area is approximately proportional to (body weight)$^{2/3}$; this will be considered later.

The results described below are based on analysis 6 for the following reasons: (*a*) the use

of log. (oxygen consumption rate) and log. (body weight) enables a test to be made of the proposition that rate of heat loss is proportional to (body weight)$^{2/3}$; (*b*) the use of rectal temperature rather than log. (rectal temperature) is based on the assumption that Q_{10} is constant over the observed range of rectal temperatures; (*c*) analysis 6, involving age, rather than analysis 5, involving log. (age), was used because this resulted in a smaller residual variance for all three parts of the investigation and there seemed to be no other grounds on which to base a choice between the two.

TABLE 2. The functions of the dependent and independent variables used in each of six multiple regression analyses, and the residual standard deviations

Analysis	Dependent variable. Oxygen consumption (ml./min)	Independent variables: Body weight (kg)	Rectal temperature (°C)	Age (hr)	Residual standard deviations* (ml./min)
1	y	x_1	x_2	x_3	3·1
2	y	$x_1^{\frac{1}{3}}$	x_2	x_3	3·1
3	y/x_1	$x_1^{-\frac{1}{3}}$	x_2	x_3	3·2
4	log y	log x_1	log x_2	log x_3	3·3
5	log y	log x_1	x_2	log x_3	3·3
6	log y	log x_1	x_2	x_3	3·2

* Adjusted to the same units for all six analyses, and pooled over the three parts of the experiment to give estimates on 86 degrees of freedom.

TABLE 3. Partial regression coefficients, and their standard errors, for log. (oxygen consumption rate, ml./min) on log. (body weight, kg), rectal temperature (°C) and age (hr)

Ambient temperature and age	No. of pigs	Log. (body weight, kg)	Rectal temperature (°C)	Age (hr)
30° C first week	61	0·610 ± 0·064	0·157 ± 0·035	0·000748 ± 0·000403
4° C first week	18	0·562 ± 0·136	0·105 ± 0·029	0·001106 ± 0·000763
30° C 1–5 weeks	19	0·684 ± 0·083	0·150 ± 0·038	−0·000372 ± 0·000126

RESULTS

The means and ranges of oxygen consumption rate, body weight, rectal temperature and age are given in Table 1. The logarithmic transformation was applied to oxygen consumption rate and body weight before doing the multiple regression analyses (see Methods); the partial regression coefficients obtained from these analyses are given in Table 3. Comparisons of the coefficients with their standard errors show that for the first week after birth the coefficients for the effect of age, after allowing for variations in body weight and rectal temperature, were not quite significant at the conventional 5% level. Taken together, however, there is a suggestion of a positive relationship. All other regression coefficients were significant.

The partial regression coefficients given in Table 3 were used to find equations for predicting oxygen consumption rate (y, ml./min) from body

weight (x_1, kg), rectal temperature (x_2, °C) and age (x_3, hr). The equations are:

$$30° \text{ C first week}: y = 0{\cdot}0442x_1^{0{\cdot}610}\, e^{0{\cdot}157x_2}\, e^{0{\cdot}000748x_3},$$
$$4° \text{ C first week}: y = 0{\cdot}7134x_1^{0{\cdot}562}\, e^{0{\cdot}105x_2}\, e^{0{\cdot}001106x_3},$$
$$30° \text{ C 1–5 weeks}: y = 0{\cdot}0598x_1^{0{\cdot}684}\, e^{0{\cdot}150x_2}\, e^{-0{\cdot}000372x_3}.$$

The percentages of variance in oxygen consumption rate accounted for by variations in log. (body weight), rectal temperature and age are given in Table 4; these factors together account for about 90% of the total

TABLE 4. The comparative effects of body weight and age as shown by the percentage of variance in log. (oxygen consumption rate) accounted for by variations in log. (body weight), rectal temperature and age taken separately and in certain combinations

	Variations in log. (body weight) allowed for first			Variations in age allowed for first		
Ambient temperature and age	log. (B.W.)	log. (B.W.) and R.T.	log. (B.W.), R.T. and age*	Age	Age and R.T.	Age, R.T. and log. (B.W.)*
30° C first week	78·8	85·7	86·4	58·7	65·1	86·4
4° C first week	82·2	93·6	94·0	84·8	87·6	94·0
30° C 1–5 weeks	69·8	83·0	88·6	10·5	40·2	88·6

* All three variables have been allowed for in both these columns and so the percentages of variance accounted for are the same. B.W. = body weight; R.T. = rectal temperature.

TABLE 5. Additional percentage of variance in log. (oxygen consumption rate) accounted for (1) by age after log. (body weight) and rectal temperature had been allowed for first, and (2) by log. (body weight) after age and rectal temperature had been allowed for first

Ambient temperature and age	(1) Additional variance accounted for by age	(2) Additional variance accounted for by log. (body weight)
30° C first week	0·7	21·3
4° C first week	0·4	6·4
30° C 1–5 weeks	5·6	48·4

variance in all three groups of pigs. The percentages of variance explained by both log. (body weight) and age are high when each is taken by itself (with one exception) since these two factors are highly correlated and the figures for each contain variance explained by the correlation. The exception is the lower figure in the 30° C 1–5-weeks pigs, which suggests a greater variation of body weight with age than in the first-week pigs.

Table 5 (derived directly from Table 4) shows that age accounts for only a small part of the log.(oxygen consumption rate) variance after log.(body weight) and rectal temperature have been allowed for, whereas log. (body weight) still accounts for a considerable part of the total variance after age and rectal temperature have been taken into account.

This distinction between the amounts of additional variance explained by age and weight cannot be made from the analysis given by Holub, Ježková & Forman (1958) of the results from their work on the oxygen consumption of the pig; in their analyses age and body weight were not considered simultaneously in a multiple regression analysis, and so they were unable to determine whether age had an effect independently of body weight.

DISCUSSION

Body weight

With increasing body weight the heat production per unit body weight decreases, whilst heat production calculated per unit surface area remains more uniform. This would apply when there is a constant deep body temperature with constant thermal conductance from body core to surface, resulting in a constant rate of heat loss per unit surface area. As the animal grows, heat production per unit body weight decreases; body surface area increases more slowly than body weight. The regression coefficients are not sufficiently precise to allow accurate estimation of the power to which body weight should be raised to give a factor proportional to the total heat production; the indices (regression coefficients) obtained for body weight in the three parts of the experiment, $0{\cdot}61 \pm 0{\cdot}06$, $0{\cdot}56 \pm 0{\cdot}14$ and $0{\cdot}68 \pm 0{\cdot}08$, do not differ significantly from 2/3, which is the appropriate index for relating volume to surface area for a solid body of a given shape but varying in size. Chiu & Hsieh (1960), in studying the metabolic rate of rats, compared the following functions: body weight, (body weight)$^{\frac{2}{3}}$, (body weight)$^{\frac{3}{4}}$, and (body weight)$^{0{\cdot}63}$; the last of these was considered to be directly proportional to surface area. None of these functions was found to be satisfactory, and Chiu & Hsieh concluded that when comparing metabolic rates of groups of animals with different average body weights analyses of co-variance should be used. They did not indicate the power to which weight should be raised in such analyses, but this question does not arise if the logarithmic transformation is applied to oxygen consumption rate and body weight. They further concluded that metabolic rate did not vary with age independently of concomitant changes in body weight, but they did not consider possible effects of variations in body temperature. The effect of ignoring rectal temperature (and the small effect of age) can be seen from the results of the present investigation on the pigs under 1 week old, where there was appreciable variation in rectal temperatures. The regression coefficient when rectal temperature and age were ignored was $0{\cdot}86 \pm 0{\cdot}05$ for these pigs (averaging over the two ambient temperatures), compared with $0{\cdot}60 \pm 0{\cdot}06$ when rectal temperature and age were allowed for.

Age

The effect of age on oxygen consumption rate, after eliminating the effects of variations in body weight and rectal temperature, was not significant for the pigs less than 1 week old but was significant for the older pigs, oxygen consumption rate decreasing with increasing age (Table 3); the difference in the partial regression coefficients between the two age groups indicates that the relation between oxygen consumption rate and age is curvilinear. The negative partial regression coefficient of oxygen consumption rate on age does not mean that oxygen consumption rate decreases as the pig grows older—indeed, the reverse is true because of increasing body weight with age—but indicates that if a number of pigs have the same body weight and rectal temperature the older pigs will have a lower oxygen consumption rate. If such animals were to be in thermal equilibrium, then heat production would equal heat loss, and, for a given core temperature, the heat loss of the individual animal would be determined by the total thermal conductance from body core to body surface. This conductance in turn is the product of the mean thermal conductance per unit area and the animal's surface area, for given surroundings in respect of radiant, convective, conductive and evaporative losses of heat.

If the mean thermal conductance were to decrease as the pig aged, a lower heat-production rate would suffice to maintain a constant rectal temperature. A developing fat layer of itself would not be expected to offer insulation in the warm surroundings of 30° C: skin blood-vessel dilatation overrides any such effect (Miller & Blyth, 1958). The development of hair, however, would allow skin temperature to remain near its previous level whilst the surface temperature of the hair itself, as an insulating layer of trapped air, would fall. This would represent an effective decrease in the rectal–air thermal conductance, and the progressive increase in hair, which occurs in the pig, would allow the maintenance of a constant rectal temperature with a progressively decreasing heat-production rate.

The total non-evaporative heat loss from the animal depends not only on the mean thermal conductance, but also on the total body surface available for heat exchange with the surroundings. Effective surface area can change rapidly, in an animal of given weight, owing to change of posture, as when the animal crouches in cold surroundings or lies extended in the warm; and surface area can change slowly, owing to change in the shape of the animal. It is possible that amongst the postulated group of pigs, all of the same weight and rectal temperature, the older ones may have a smaller surface area owing to change in body conformation and fat deposition.

The most likely reasons for the fall in oxygen consumption with age,

with body weight and rectal temperature held constant, would therefore be the decrease in total thermal conductance brought about by the growth of hair, and the possible reduction in the surface area effective for heat loss at any given body weight. In postulating possible causes of this negative partial regression of oxygen consumption on age, it may be re-emphasized that the changes involved must be related to age independent of body weight. In considering the development of hair as a possible explanation, therefore, it is not enough that hair develops as the pig grows larger; the amount of hair must be greater with old pigs than with young pigs of the same body weight (and the same rectal temperature).

Rectal temperature

The partial regression coefficients for rectal temperature (Table 3) give the values for Q_{10} directly. Thus, this coefficient is $0{\cdot}157 \pm 0{\cdot}035$ for the 30° C first-week pigs, and 0·157 is the natural logarithm of the difference in oxygen consumption rate for 1° C. 1·57 is therefore the corresponding difference for 10° C, and antilog$_e$ 1·57 is 4·81, which is the Q_{10}. The mean values for Q_{10}, with their associated 95% confidence intervals, are as follows:

30° C first week: 4·81 (2·39 to 9·74)
4° C first week: 2·86 (1·54 to 5·31)
30° C 1–5 weeks: 4·48 (2·01 to 10·03)

The usual figure given for animal tissues, from both poikilotherms and homoeotherms, is between 2 and 3 (e.g. Valen, 1958, for poikilotherms; Graham, Wainman, Blaxter & Armstrong, 1959, for the conscious sheep), similar to that for chemical reactions. The high values obtained for the conscious pig in the present experiments, where most of the observations were made at temperatures below the critical temperature, suggest that in the postulated group of pigs of the same weight and age those with the higher body temperatures have higher metabolic rates not only as a result of the effect of temperature on metabolic processes, but also because the higher rectal temperature is correlated with other factors, including possibly the level of spontaneous activity, such as standing instead of lying in the calorimeter chamber. This would probably have a proportionately smaller effect with the higher metabolic rates of pigs at lower ambient temperatures which could account for the tendency to a lower Q_{10} at 4° C. If it is true that animal behaviour patterns seriously affect Q_{10}, the concept is of limited value when applied to metabolic rates of conscious animals.

Other species

New-born mammals range from apparent poikilothermy to homoeothermy in their metabolic response to change of environment in the post-

partum period. The mouse (Fitzgerald, 1953) goes through a period immediately after birth in which it is poikilothermic, without increased metabolism in response to cooling of the surroundings. Adolph (1957) has discussed the development of homoeothermy in the rat, and this discussion, together with other evidence (Čapek, Hahn, Křeček & Martinek, 1956) makes it clear that age is a very important factor in the animal's development since it represents the time, particularly during the first 3 weeks, when nervous control is developing and allowing the animal a full metabolic response to changes in its surroundings. In the case of the puppy McIntyre & Ederstrom (1958) have shown that heat production increases over the first 2 weeks of life, but that heat conservation lags behind. This lag in the development of heat conservation behind heat production occurs in the case of the pig as well; in cool surroundings the new-born pig's rectal temperature falls, but this effect largely disappears by the end of the first week (Mount, 1958). Thermogenesis in the pig is, however, well developed at birth; this is shown by the vigorous metabolic response to short-term cooling of the environment (Mount, 1959) and the small effect of age indicated by the present work. In the dog Gelineo (1954) found the thermo-neutral temperature to fall from near 33° C at birth to near 30° C at 2–3 weeks of age; a similar reduction in critical temperature in the first weeks of life also takes place in the pig (Mount, 1960). An animal born in an advanced state, such as the calf (Roy, Huffman & Reineke, 1957) and lamb (Dawes & Mott, 1959), shows a high rate of heat production from birth, like the pig. In addition, in the case of the calf and lamb the thick coat adds considerably to the extent to which the animals can conserve heat, giving them that advantage in homoeothermic development over the pig.

SUMMARY

1. Measurements of oxygen consumption have been made in a closed-circuit apparatus on 98 pigs aged from 1 hr to 37 days. The measurements have been divided into three groups depending on the ambient temperature at which the observation was made and the age of the pig: 30° C first week, 4° C first week and 30° C 1–5 weeks.

2. The relation between oxygen consumption rate and body weight, rectal temperature and age has been examined by means of multiple regression techniques, which allow the relation of each of these variables to be assessed separately while the other two are held constant.

3. In the first 5 weeks after birth body weight and rectal temperature both had a large effect on oxygen consumption. The effect of age, after allowing for differences in body weight and rectal temperature, was less striking. Indeed, during the first week it was not certain whether it had

any effect at all; from 1 to 5 weeks metabolic rate and age were negatively related.

4. The percentages of variance in metabolic rate accounted for by variations in body weight, rectal temperature and age were 86·4 % in the 30° C first-week group, 94·0 % in the 4° C first-week group and 88·6 % in the 30° C 1–5 weeks group.

5. The small effect of age, when taken with other evidence, suggests that thermogenesis in the pig is well developed at birth.

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THE HISTAMINE CONTENT OF THE HUMAN UTERUS

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A number of drugs commonly used to accelerate labour, such as morphine, pethidine and castor oil, are capable of releasing histamine (Feldberg & Paton, 1951; Schachter, 1952) and the question arose whether their usefulness and that of other tocagogues (Mathie & Dawson, 1959) depended on this action. It seemed unlikely that a gross systemic histamine release could be concerned, but a local release in uterine tissue deserved consideration. Information was not available, however, as to the amount of histamine in human uterine tissue, and the following investigation was made to determine this.

METHODS

The content of histamine was estimated in tissues from non-pregnant uteri removed at operation and in slips of tissue obtained from pregnant uteri at Caesarean section. The tissue was cut from the anterior wall and cervix of the non-pregnant uteri, from the margin of the incision of the lower segment of uteri at or near term, and from the fundus of other uteri earlier in pregnancy. The sample was blotted free from blood with sterile dry swabs, placed in a sterilized dry glass pot with an airtight lid, and stored at 0–4° C until extracted. In one case a whole uterus was available and a detailed study of the distribution of histamine in it was made. Tissue was also obtained from both the pregnant and the non-pregnant horns of one bicornuate uterus. The tissue was ground in acidified NaCl solution, 0·9 g/100 ml., with acid-washed sand, boiled and filtered, by the method used by Feldberg & Paton (1951). The extracts were assayed on the guinea-pig ileum, using mepyramine 10^{-8} or 10^{-9} to verify that the contractor activity was due to histamine.

RESULTS

The mean value of the histamine content (as base) of the body of the non-pregnant uterus was $8{\cdot}58 \pm 1{\cdot}60$ μg/g fresh tissue and of the cervix $5{\cdot}97 \pm 1{\cdot}22$ μg/g (Table 1). The content was found to vary from one part of the body of the uterus to another (Fig. 1) but this variation had no obvious significance in its pattern.

The content of the pregnant uterus was much lower, with a mean value of $0{\cdot}93 \pm 0{\cdot}22$ μg/g. The site of sampling depended on whether the classical Caesarean or a lower-segment operation was done; but this did not materially influence the histamine content. The values were of the same order in

tissue from four patients who had been in labour before Caesarean section as in those on whom an elective operation was performed before labour began; nor did the values obtained in earlier pregnancy at 14, 22, 30 and 33 weeks differ from those obtained at term.

There was no evidence of activity attributable to 5-hydroxytryptamine in any of the samples.

TABLE 1. Histamine content of human uterine tissue (μg/g)

(*a*) Non-pregnant

Case no.	Fundus	Cervix	Remarks
1	4	5	Progesterone 25 mg previous day
2	6·4	11·2	—
3	5	—	—
4	10	2·4	—
5	3·5	9	Ethynyloestradiol previous day
6	14	3	—
7	15	5·3	—
8*a*	11·7	—	Bicornuate uterus: non-pregnant horn
Mean ± S.E.	8·58 ± 1·60	5·97 ± 1·22	

(*b*) Pregnant

Case no.	Fundus	Lower segment	Weeks of pregnancy	
8*b*	—	3·5	40	Bicornuate uterus: pregnant horn. Pitocin and ergometrine between samples 8*b* and 8*c*
8*c*	—	1·9		
9*a*	—	< 0·2	40	Ergometrine between samples 9*a* and 9*b*
9*a*	—	0·03		
10	—	1·4	40	
11	—	< 1	40	
12	—	0·3	39	
13	—	0·31	39	
14	—	0·50	39	
15	—	0·21	38	
16	—	0·74	36	
17	1·5	—	33	
18	1·5	—	30	
19	0·22	—	22	
20	0·1	—	14	
Mean	0·83	0·92		

(*c*) In labour

			Hours in labour
21	< 0·5; < 0·4	40	12
22	0·88	39	31
23	0·2	44	12
24	1·1	41	65
	Mean 0·66		

DISCUSSION

Both the significance and the origin of the difference in histamine content of the pregnant and non-pregnant uterus are obscure. The values early in pregnancy, since they resemble those at term, show that the reduction

of histamine content is not only, if at all, brought about by the enlargement of the uterine muscle reducing, for instance, the relative proportion of the mast cells. On the other hand, the contrast between the two horns of the bicornuate uterus shows that the reduction in the amount of contained histamine which occurs in pregnancy is a property of the pregnant state of the uterus and not of the general systemic changes in the mother.

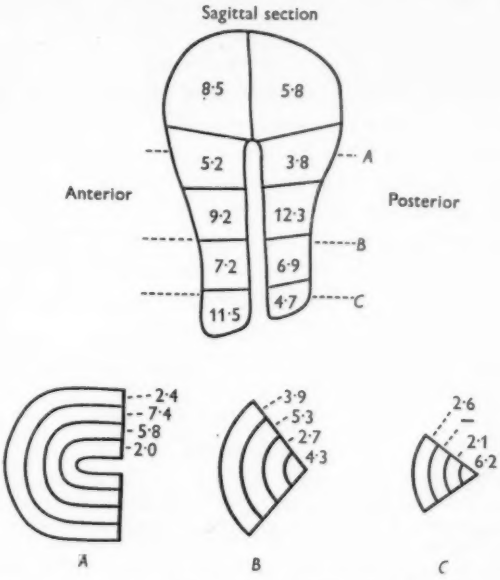

Fig. 1. Diagram of histamine content of human uterus. Above, sagittal section; below, horizontal sections at levels *A*, *B* and *C* shown above; numbers indicate histamine content (μg/g).

Kahlson, Rosengren & Westling (1958), studying rats on a synthetic histamine-free diet, found that the excretion of histamine rose steeply in pregnancy, falling the day before parturition. The rate of formation of histamine in the pregnant rat was nearly three times the rate before mating and the daily urinary output was greater than the content of the whole body. In further experiments (Kahlson, Rosengren & White, 1960) the foetal liver was found to be the site of extremely fast formation of histamine. The same workers report a high rate of synthesis of histamine in the human foetus (Kahlson, Rosengren & White, 1958). The findings in the rat emphasize the difficulty of interpreting the kinetics of histamine formation from the histamine content of a tissue. It cannot be inferred, for instance, that the low histamine content of the pregnant uterus proves

that histamine plays no part in uterine activity at pregnancy; but it makes it somewhat unlikely that local histamine release is involved in the induction of labour. It is possible, however, that the high serum histaminase of pregnancy in the human being and the low histamine content of the pregnant uterus are concomitants of an increased sensitivity of the pregnant uterus to histamine, allowing a restricted degree of histamine release from a source other than the uterus to become effective.

SUMMARY

1. The histamine contents of the human non-pregnant and pregnant uterus have been estimated from samples removed at surgical operation.

2. The content in the pregnant uterus (mean, 0·83 μg/g) was considerably lower than (only about one-tenth) that of the non-pregnant (8·58 μg/g) and this difference was also found between the pregnant and non-pregnant horns of a bicornuate uterus.

3. The values found in the uterus during pregnancy (14–40 weeks) did not differ significantly from those at term, nor from those obtained when the subject had been in labour.

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ERRATA

LINZELL, J. L. (1960). *J. Physiol.* **153**, 510–521.

Figures for lactate should read as follows (m-equiv/l.):

p. 513, Table 1: 1·36 ± 24, 1·56 ± 0·2, 0·88 ± 0·22, 0·7 ± 0·8, 0·4 ± 0·1 (9);

p. 517, l. 32: 1·8 and 2·4.

p. 520, l. 11: *for* '2 and 12' *read* '7 to 14'.

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for MAUNG PE THEIN and BRENDA M. SCHOFIELD

read MAUNG PE THEIN and B. SCHOFIELD